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ESTIMATION OF HISTAMINE IN THE BLOOD AND OTHER TISSUES OF RATS AND GUINEA PIGS INFECTED WITH *TRICHINELLA SPIRALIS*[1]

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Although the life cycle of *Trichinella spiralis* (Owen) is well understood and the histopathology and immunology of trichinosis have been extensively investigated, little is known concerning the toxicology of the infection. Since one of the products of tissue destruction is believed to be histamine, and the acute symptoms of trichinosis appear at the height of tissue invasion and presumably tissue destruction, an investigation has been made concerning changes in the histamine content of the blood and certain other tissues of albino rats and guinea pigs experimentally infected with *T. spiralis*.

Several investigators have suggested the possibility that the destruction of tissue and irritation caused by the migrating larvae of *T. spiralis* might result in the production of toxic materials and chemical changes in the blood. The first attempt to detect such poisons and determine their chemical nature was made by Flury (1913), who reproduced the symptoms of trichinosis by the subcutaneous injections of aqueous and alcoholic extracts of heavily infected muscles. He attributed the effects to no one particular substance but rather to a group of purine bases and creatine derivatives, such as methyl guanidine. Harwood, Spindler, Cross and Cutler (1937) using the method of Minot and Dodd (1933), reported an increase in guanidine in rabbits infected experimentally with *T. spiralis*. While increased guanidine has been reported in several diseases, its significance has been questioned (Sollman, 1942).

Histamine has a more pronounced pharmacological action than does guanidine and may possibly be a significant toxic agent in trichinosis. Increases of this substance in the blood and other tissues have been reported in a number of conditions including severe burns (Rosenthal, 1937), anaphylactic shock (Code, 1939), traumatic shock (Dale and Laidlaw, 1919), and adrenalectomy (Rose and Browne, 1941).

PROCEDURE

The strain of *Trichinella spiralis* used in the present study was carried in albino rats to provide larvae for experimental infections. Rats were fed ground infected

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muscle tissue while guinea pigs were infected with larvae from artificial digests, administered by stomach tube. Rats were given doses of from 600 to 10,000 larvae, and guinea pigs 1,000 to 3,000. After considerable experimentation, 6,000 larvae per rat and 3,000 per guinea pig were found to be satisfactory for producing symptoms of trichinosis. Whenever possible, litter mates were employed in each series. Symptoms were more uniform in guinea pigs than in rats, supporting Roth's (1939) opinion that the guinea pig is the better animal for experimental trichinosis. Since animals are sometimes resistant to infections with *T. spiralis,* the diaphragms of all experimental rats and guinea pigs were examined microscopically before proceeding with assays for histamine. Most of the histamine determinations were made on rats 16 to 18 days after larvae were administered, and on guinea pigs, 23 to 26 days.

Histamine in the blood was determined by the method of Barsoum and Gaddum (1935) as modified by Code (1937); other tissues, including lung, liver, intestine, skeletal muscle and kidney, were extracted according to the method of Best and McHenry (1930) as modified by Rose and Browne (1941). All assays were carried out on segments of isolated guinea pig ileum removed from animals that had been starved 24 hours. Refrigerated segments, as used by Minard (1941), were unsatisfactory because they were not sufficiently sensitive to the concentrations of histamine involved. The test strip was exposed to a standard histamine hydrochloride until a maximum contraction was obtained (never more than 30 seconds), then washed for 30 seconds in Tyrode's solution, permitted to rest 30 seconds, exposed to a standard or extract until a maximum contraction was obtained, then washed with saline, and the assay repeated in the same sequence. This order was rigorously maintained during the assay to obtain consistent contractions of the intestinal segment. Several determinations were made on each extract before it was discarded.

Rosenthal's (1937) method utilizing small amounts of whole blood was tried with a number of infected animals in an attempt to follow changes in histamine content as the infection advanced.

Differential white cell counts were made on a number of animals, using Giemsa stained smears. No attempt was made to differentiate the various types of lymphocytes.

RESULTS

Attempts to Estimate Blood Histamine Content by the Whole Blood Method of Rosenthal

Attempts were made to determine the blood histamine in 69 rats and 18 guinea pigs by assaying 2–4 per cent suspensions of whole blood in saline on the isolated ileum of the guinea pig. It was found that slight contractions of the intestinal segment resulted but consistent quantitative determinations could not be obtained and there was a rapid loss of activity. There was some indication that a substance or substances, which caused the contraction of the ileum, did increase in infected animals. The method gave such erratic results, however, that its use was discontinued.

Estimation of Blood Histamine by Code's Modification of the Extraction Method of Barsoum and Gaddum

The trichloracetic acid extraction method was employed to determine the blood histamine of 25 experimental and 23 control rats and 21 experimental and 21 control guinea pigs.

The results obtained with experimental and control rats are shown graphically in Fig. 1. Histamine values for the blood of control rats ranged from amounts too small to be detected to 3.5 gamma/ml, with a mean of 0.5 gamma/ml. In the infected animals, the values ranged from 0.04 to 5.0 gamma/ml of blood, with a mean of 1.4 gamma/ml. Seventeen of the infected rats showed an increase in blood histamine over the mean value obtained for control animals, while eight gave histamine values equal to or less than the mean for controls.

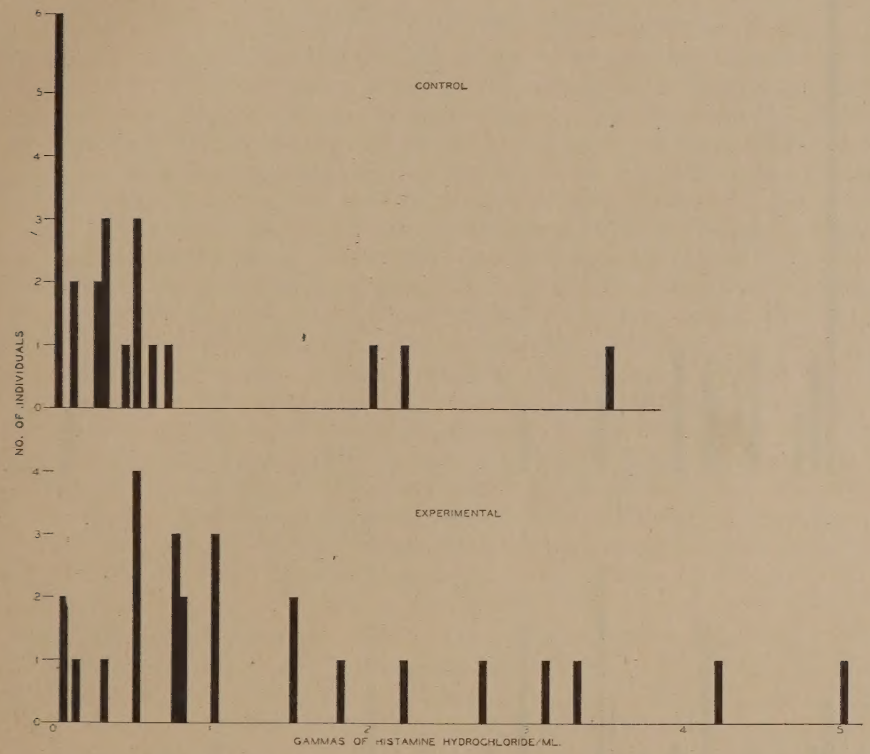

FIG. 1. Blood histamine of rats. Experimental animals infected with approximately 6000 larvae of *Trichinella spiralis* and assayed 16–18 days after infection.

The results obtained with experimental and control guinea pigs are shown in Fig. 2. The histamine content of the blood of the control guinea pigs varied from undetectable amounts to 2.0 gamma/ml with the exception of one animal which gave an unusually high value of 5.0 gamma/ml. The mean for control guinea pigs was 0.7 gamma/ml. Blood histamine values for infected guinea pigs ranged from undetectable amounts to 4.0 gamma/ml with an average value of 1.5 gamma/ml. Seventeen of the infected guinea pigs gave blood histamine values in excess of the mean obtained for the control animals while four gave histamine values equal to or less than the mean for controls.

Determination of Histamine in Tissues other than Blood

In six experimental rats, the histamine content of lung tissue varied from 1 to 12 gamma/gm with a mean of 4.0 gamma/gm and in seven control rats the range was from 3 to 10 gamma/gm with a mean of 5.3 gamma/gm. In 13 experimental guinea pigs, the lung histamine value ranged from amounts too small to be detected to 14 gamma/gm with a mean of 4 gamma/gm while 13 control guinea pigs gave values

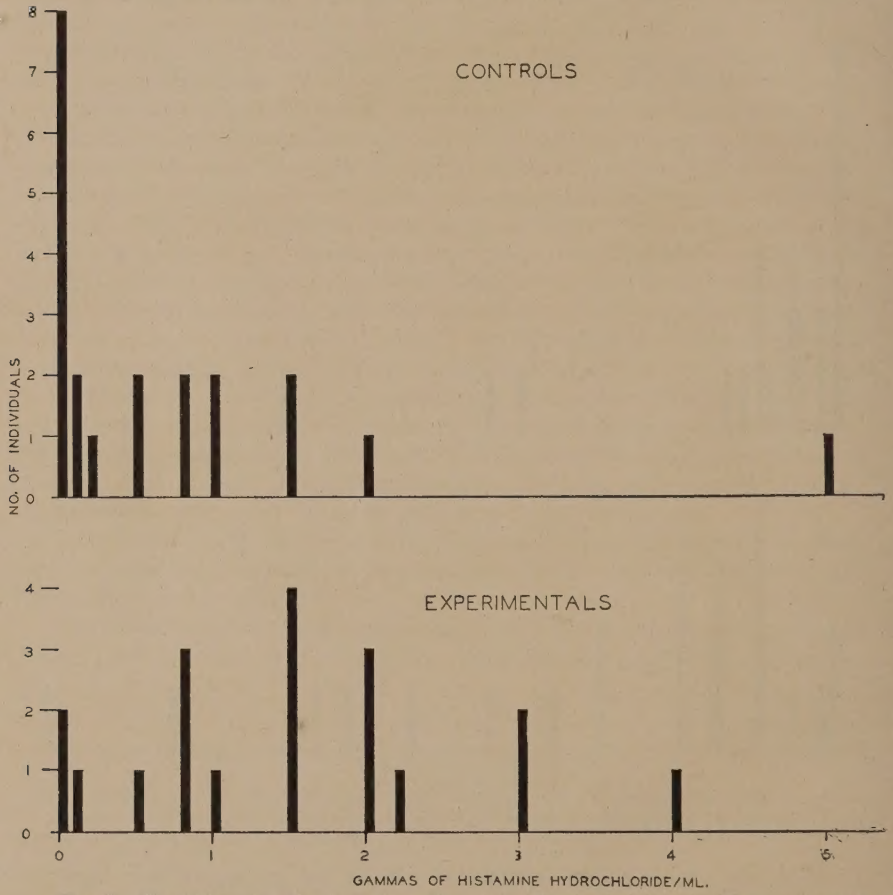

FIG. 2. Blood histamine of guinea pigs. Experimental animals infected with approximately 3000 larvae of *Trichinella spiralis* and assayed 23–26 days after infection.

ranging from undetectable amounts to 6 gamma/gm with a mean of 2.3 gamma/gm. While variation in respect to lung histamine is greater in experimental rats and guinea pigs than in control animals, mean values indicate no significant difference. Histamine content of lung tissue did not seem to be correlated with that of the blood from the same animal.

No significant differences were noted in the histamine content of liver, kidney,

and skeletal muscle of experimental and control rats and guinea pigs. Differences were noted, however, in intestinal tissue of guinea pigs when experimental animals showed marked inflammation of the duodenum as sometimes occurs in acute trichinosis. The intestine of control guinea pigs gave values of 1.0 gamma/gm or less while that of infected guinea pigs with duodenal inflammation gave values up to 8.0 gamma/gm. This difference appears to be significant but the number of animals showing duodenal inflammation was too small to warrant definite conclusions.

Observations on Differential Blood Counts

The results of differential blood counts on experimental and control rats agree fairly well with those reported by Beahm and Downs (1939). Lymphocyte counts on 34 infected rats varied from 48.4 to 84.5 per cent, with a mean of 68.8 per cent, while those of 20 controls varied from 58.5 to 89.5 per cent with a mean of 78.8 per cent. In experimental rats, neutrophile counts varied from 13.4 to 50.3 per cent, with a mean of 24.6 per cent; controls varied from 6.9 to 27.6 per cent, with a mean of 17.1 per cent. There was no appreciable difference between basophile counts in experimental and control rats, the values in both groups ranging from 0.0 to 0.6 per cent. A slight eosinophilia was observed in some of the infected rats, the count ranging from 0.0 to 23.4 per cent, with a mean of 6.4 per cent. Control rats showed eosinophile counts of from 0.6 to 10.8 per cent, with a mean of 4.1 per cent.

Similar results were obtained with guinea pigs. However, eosinophilia in infected animals was more consistent than in rats. Eosinophile counts on 30 experimental guinea pigs varied from 0.3 to 44.8 per cent, with a mean of 10.9 per cent, while 18 control animals gave counts of from 0.0 to 22.6 per cent, with a mean of 4.0 per cent. In both rats and guinea pigs, there did not seem to be a consistent correlation between the eosinophile counts and blood histamine values; some animals with severe symptoms and high blood histamine values gave very low eosinophile counts.

DISCUSSION

Mean values, based upon the above data, indicate that there is an increase of histamine in the blood and possibly certain other tissues of rats and guinea pigs infected with *Trichinella spiralis*. Although the formation of this substance might be expected as a result of the irritation and destruction of tissue by migrating larvae, it may be that the presence of the worms and their metabolites would produce an anaphylactoid condition with concomitant increases in histamine. Code (1939) has found that there is a marked increase in the blood histamine of dogs and guinea pigs during anaphylactic shock.

The results of the present study are difficult to evaluate because of the variability of both experimental and control animals. Furthermore, there is little information concerning the pharmacological significance of slight changes in the histamine content of the blood and tissues and the rapidity with which it may be excreted or destroyed and thus influence the concentration that would be detected at the time of assay.

A comparison of histamine poisoning and trichinosis reveals several interesting parallels. In both conditions, various investigators have reported cardiac insufficiency and lowered blood pressure, edema, inflammation and ulceration of the intes-

tine (in trichinosis, in regions in which adult worms are absent), acidosis and eosinophilia. However, some of these observations have been reported only once or a few times and, in certain cases, have not been confirmed by other investigators.

SUMMARY

The histamine content of the blood and certain other tissues of rats and guinea pigs infected with *Trichinella spiralis* has been investigated. Infected animals showed greater variability than controls but gave higher mean values for blood histamine. Observations on lung, kidney, skeletal muscle and intestinal tissue gave less conclusive results except in the intestinal tissue of infected animals in which inflammation of the duodenum was pronounced.

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A FURTHER DESCRIPTION OF *STEMPELLIA MONIEZI* JONES, 1942, A MICROSPORIDIAN PARASITE (NOSEMATIDAE) OF CESTODES

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Kudo's monograph on the Microsporidia (Kudo, 1924), lists "Gen. incertum *helminthophthorum* (Keferstein)" under "ambiguous forms" with the following synonymy: *Mucor helminthophthorum* Keferstein, 1862; *Nosema helminthophthorum* Moniez, 1887; *Plistophora helminthophthora* Labbé, 1899. As this list summarizes a rather interesting historical sequence, it may serve as a starting point for an analysis of the earlier reports of microsporidia from cestodes.

REVIEW OF LITERATURE

Bischoff (1855) without naming, described in some detail what was probably a microsporidian from the nematode, *Ascaris mystax*. The dimensions of the spore (which, he said, was motile, and possessed a "keinerlei fadenförmigen Anhang") he gave as circa 8 by 4 micra. Munk (1857) added little but considered the form, which he also found in *Ascaris mystax*, to be identical with that of Bischoff, although non-motile, thus questioning Bischoff's interpretation. Munk did, however, foreshadow later conclusions by Moniez and others, in comparing the effect of the parasite upon the host with the symptoms of the then recently described silkworm disease. Although credit for an original description has been given by subsequent writers to Keferstein (1861), he described, in the present author's opinion, no protozoan parasite at all, but perhaps a fungus, as he and de Bary thought.[2] His figure, of a hyphate structure bearing "sporangia" at the tips of its many branches, is hardly recognizable as the gross structure of a microsporidian, and indeed resembles strongly a water-mold. Moniez (1887), however, considered that Keferstein, Bischoff, and Munk (l.c.) had all seen the same form. He considered this form to be identical with or very close to his *Nosema helminthophthorum*.[3] Moniez, by placing this form in the genus *Nosema* Nägeli, to which the causative agent of the pebrine disease belongs, supported Munk's observation that probably the disease of the nematode, *A. mystax*, was related to the disease of silkworms. Labbé (1899) transferred Moniez's parasite to the genus *Plistophora* Gurley, giving the spore dimensions as 4.2–5.9 by 1.7–2.5 micra. He described "vesicles de 20 μ avec

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[1] The author gratefully acknowledges the helpful suggestions and advice of Dr. Bruce D. Reynolds of the Miller School of Biology, University of Virginia, under whose direction this work was carried out.

[2] De Bary, to whom Keferstein submitted his material, wrote to Keferstein that "der vorgelegte Pilz zur Gattung Mucor Sect. Hydrophora gehörte und dass er ihn Mucor helminthophthorus benennte. Herr de Bary bemerkt weiter, dass dieser Pilz viele Ähnlichkeit mit einen von H. Hoffmann aus dem Chylusmagen der Biene beschrieben und Mucor melitophthorus benannten Pilz habe."

[3] He said (p. 1312) "cette espèce vit chez certains Taenias inermes et je l'ai indiquée des 1879, les spores du parasite s'observent en énorme quantité dans les mailles des tissus; elles pénètrant á l'interieur des ovules dont elles n'empêchent pas toujours l'evolution, et c'est ainsi qu'elles passent á le nouveaux hôtes; ces spores sont ovales et measurent prés de 5 μ sur 2 μ, 5; elles present tous les caractères optiques et chemiques du parasite de la pébrine; . . . ."

pellicule," and gave a host list to include the "Taenias inermes" of Moniez, and *Ascaris mystax* of Bischoff, Munk, and Keferstein.

The attempts by the foregoing authors, especially Moniez, Labbé, and Kudo, to bring together the several descriptions of sporeforming parasites of nematodes and cestodes into one genus do not seem very successful, unless the discrepancies (as shown plainly in Table 1) be dismissed as due to faulty observation. There appears

TABLE 1.—*Comparison of data given in cited reports of microsporidia from cestodes and a nematode*

Name	No. spores per sporont	Spore size (micra)	Host	Location
Not given ..... (Bischoff, 1855)	?	7.7 by 4.4	*Ascaris mystax* (nematode)	Seminal vesicles; female genital system
Not given ..... (Munk, 1857)	?	4.2–5.9 by 1.7–2.1	Same	Same
Mucor helminthophthorus ... (Keferstein, 1861)	?	4–5 by 2	Same	Genital system and intestine; hyphate, with sporangia
Nosema helminthorum ..... (Moniez, 1887)	?	5 by 2.5	"Certains taenias inermes"	Tissues, ovaries, ova; in great masses
Plistophora helminthophthora (Kef.) (Labbe, 1899)	?	4.2–5.9 by 1.7–2.5	**Taenia expansa* Rud. **T. denticulata* Rud. **T. bacillaris* Goeze	"Parenchyme, organs genitaux, ovules"
Stempellia moniezi ..... (Jones, 1942)	1, 2, 4, or 8	4–5 by 1.5–2.0	*Hymenolepis anthocephalus* van Gundy *Diorchis reynoldsi* Jones, 1943	Parenchyma, mainly in region of excretory canals

* According to Hughes (1941), *Taenia expansa* Rud. = *Moniezia expansa* (Rud.) Blanchard, 1891. *T. bacillaris* Goeze, 1782 = *Hymenolepis bacillaris* (Goeze) Blanchard, 1891. *T. denticulata* Rudolphi, 1810 = *Cittotaenia denticulata* (Rud.) Stiles, 1896.

to be no compelling reason for so dismissing the data of Keferstein and the others; and the author begs leave to suggest that the dismissal of the data of other workers, in order to bring credence to one's own generalization, is at best a practice rather damaging to scientific accuracy. In preference, therefore, to the treatment by Kudo (although, in fairness, his listing of the parasites concerned under "genus incertum helminthophthorum" does not imply his absolute intention of identifying as one form the several listed), the author proposes to let the parasite described by Keferstein remain in the hands of the mycologists as *Mucor helminthophthorus* Keferstein, 1862, to be treated by them as they wish. The other descriptions of the parasites of *Ascaris mystax* and several cestodes (see Table 1), although probably correct as to sub-class and family designation, are inadequate as far as genus and species are concerned. None of them gives the essential information, the number of spores formed from one sporont. Details of spore structure and sporogenesis are lacking. It is therefore impossible to determine whether all or any of the earlier reports actually refer to the present species. In recognition, however, of the fact that Moniez first combined name and description for a microsporidian parasite of cestodes, the present species was named in his honor (Jones, 1942).

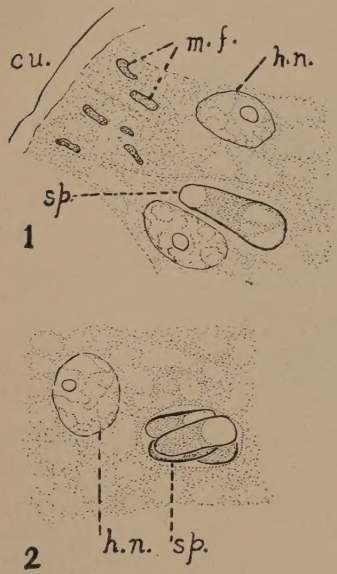
GENERIC ALLOCATION

In the genus *Nosema,* in which Moniez placed his "species," one sporoblast gives rise to one spore only. In the genus *Plistophora,* to which Labbé transferred

Moniez's species, the number of spores per sporoblast is sixteen or more. In the genus *Stempellia,* the number of spores per sporoblast is characteristically one, two, four, and eight, all four numbers being found in a single infection (see Kudo, 1924). Since by the description following, the present species is shown to produce one, two, four, or eight spores, per sporoblast, it seems plain that the species should be placed in the genus *Stempellia* Leger et Hesse, 1910.

Stempellia moniezi Jones, 1942
(Figs. 1, 2)

Specific diagnosis: Sporozoa of order Microsporidia Balbiani, 1882, suborder Monocnidea Leger et Hesse, 1910, family Nosematidae Labbé, 1899, and genus *Stempellia* Leger et Hesse, 1910; in parenchymatous syncytium of *Hymenolepis anthocephalus* van Gundy, 1936, and



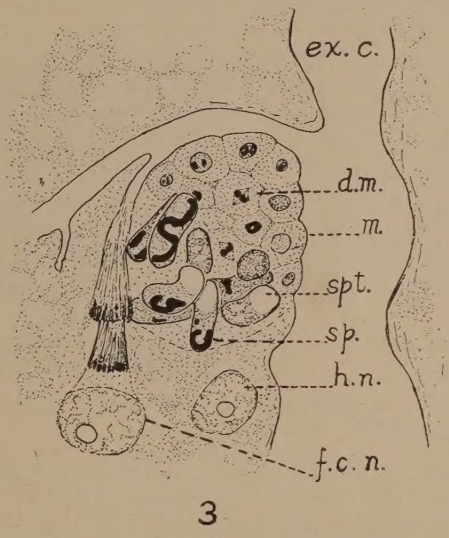


Fig. 1. Spore from monosporous sporont, lying within cytoplasm of a "sub-cuticular cell" of *Diorchis reynoldsi.* (Allen's B–15, iron haematoxylin.)

Fig. 2. Spores from tetrasporous sporont, lying in parenchyma of *D. reynoldsi.* Allen's B–15, iron haematoxylin.)

Fig. 3. Groups of meronts (some in division), sporonts, and young spores, lying against wall of ventral excretory duct of *Hymenolepis anthocephalus.* Flame cell cilia and nucleus shown at left, main canal at right. (Flemming's medium solution, crystal violet stain.)

Abbreviations: *cu.,* cuticle; *d.m.,* dividing meront; *ex.c.,* excretory canal; *f.c.n.,* flame cell nucleus; *h.n.,* host nucleus; *m.,* meront; *m.f.,* host muscle fibers; *sp.,* spore; *spt.,* sporont.

All drawings made with the aid of a Zeiss camera lucida, and enlarged 3,600 times.

Diorchis reynoldsi Jones, 1943, cestodes from the shrew *Blarina brevicauda* Say; spores thin-walled, ovoid to sub-cylindrical, 4–5 μ by 1.5–2.0 μ; meronts 2.5–3.0 μ, sub-spherical, cytoplasm clear, nucleus single; schizogony produces groups of several to many sporonts; each sporont produces one, two, four or eight spores; spores from octosporous pansporoblast smaller than those from tetrasporous or other sporoblast.

Locality: Charlottesville, Virginia, U. S. A.

Host: Hymenolepis anthocephalus van Gundy, 1936; *Diorchis reynoldsi* Jones, 1943.

Type specimens: Slides in author's collection at Miller School of Biology, University of Virginia, University, Va.

MATERIALS AND METHODS

While working in 1941 on a cytological problem concerning certain hymenolepidid cestodes, the author noted, in two cestodes from the small intestine of different specimens of the shrew *Blarina brevicauda* Say, numerous subcylindrical bodies, distinct, in form and staining qualities, from the cestode tissues. Cestodes from about fifteen shrews were examined, but no others infected with protozoa were found.

One of the infected cestodes, *Hymenolepis anthocephalus* van Gundy, 1936, was fixed in Flemming's solution (medium) and stained for karyological contrast with Newton's crystal violet iodine. The other cestode, *Diorchis reynoldsi* Jones, 1943, was fixed in Allen's B–15, stained with iron haematoxylin, and decolorized with picric acid. Sections of all material were cut at 14 micra. Externally, and largely internally, the parasites by both methods showed similar structure. Those stained

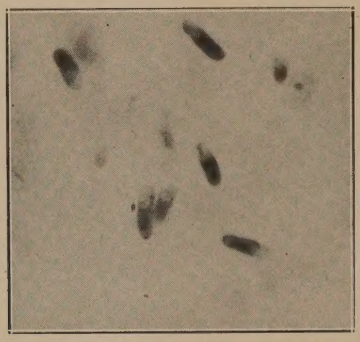


FIG. 4. Photograph of spores lying in the parenchyma of *Hymenolepis anthocephalus*. Magnification circa 1400×.

with crystal violet showed more clearly the dense fragments or filaments within the spore (see Figs. 2, 3), while those stained with haematoxylin showed spore vacuoles and cytoplasm more plainly (see Figs. 1, 2). Both preparations showed rather well the developmental stages.

OBSERVATIONS

Locality.—The parasites were found in cestodes from two short-tailed shrews in the vicinity of Charlottesville, Virginia.

Hosts.—The cestode hosts are *Hymenolepis anthocephalus* van Gundy, 1936, and *Diorchis reynoldsi* Jones, 1943. Of about thirty cestodes from shrews examined, including, in addition to the above cestodes several specimens of *Protogynella blarinae* Jones, 1943, only two cestodes were found infected. The manner of infection of the cestode host is not clear, but the possibility of penetration of the cestode cuticula by the sporozoites from spores lying in the mammalian host intestine (perhaps liberated there from the bodies of eaten insect larvae, common hosts of microsporidian parasites) is suggested by Thorne's report (1940) of penetration of a nematode's cuticle by a nosematid parasite, *Dubosqia penetrans* Thorne, 1940. It is likewise possible that ingress into the cestode tissue is by way of the excretory canals, which open in the last proglottid of the cestode. Moniez' (1887) suggestion,

that infection of new cestode hosts by microsporidia occurs by penetration of the ova, is not substantiated by any observations upon the new material.

Habitat.—Meronts, sporonts, and spores are found chiefly in the cytoplasm of the host syncytium surrounding the main excretory canals and their contributing ducts. Often, as in Fig. 3, the parasite is seen lying against the wall of these ducts. In the more heavily infected of the two hosts observed, complete invasion of the whole parenchyma was seen; yet the manner of distribution of the spores in the scolex region corresponded well with the location of the excretory tubules.

Developmental forms.—In general, the parasite conforms to the characters of the genus *Stempellia* Leger et Hesse, 1910, as defined by Kudo (1924). The meronts, about 2.5–3.0 micra in diameter, are sub-spherical uninucleate forms occurring in large or small groups resulting from schizogony. At no stage, either meront or spore, are cyst walls of any sort developed, although a differentiating line, like the "wall" of a vacuole, may sometimes be discerned between the host cytoplasm and a group of parasites. No definite shape is assumed by a group of meronts; they generally, as in Fig. 3, conform to the limiting membranes of the host.

The sporonts are a little larger than the meronts, from which they are distinguishable by a somewhat larger and less dense nucleus, and somewhat denser cytoplasm. Each gives rise to one, two, four, or eight sporoblasts. In a large group of parasites meronts, sporonts, sporoblasts, and spores can be seen; in a small group, say, of four or eight cells, all cells are of the same type, either meronts, sporonts, sporoblasts, or spores. It is probable that the large groups are "mixed"; i.e., not descended from one meront; while the small groups should be interpreted as the results of one cell's schizogony.

Spores.—The spores, one, two, four, or eight per sporont, show a slight but observable gradation in size. One individual of a double spore is smaller than the single spore, and one from a group of eight is the smallest. Average spore dimensions are: length circa 4–5 micra by width circa 1.5–2.0 micra. The spores are ovoid to subcylindical. The wall is quite thin, but appears both resistant and fairly rigid. A vacuole at one end (usually the smaller, see Fig. 1) with a vacuole-like space containing cross-strands (see Fig. 4), or a curled, twisted, or fragmentary body lying in the other, suggests plainly that the spore is similar to the typical microsporidian spore of the family Nosematidae Labbé, 1899. That is, the spore probably contains a central or subterminal "polar capsule" (the fragments of which are seen in the spores of Fig. 3) with a peripheral girdle of cytoplasm, the sporozoite. Unfortunately, the scarcity of material, and the fact of discovering the protozoa after exhausting the available fresh material, made special techniques for demonstrating a polar filament impracticable. It is possible, indeed, that complete spore development is inhibited in the cestode host, so that only partial differentiation of the filament apparatus occurs. The infrequent occurrence in the literature of reports of microsporidia from cestodes indicates an accidental host-parasite relationship, which may, therefore, be imperfect.

SUMMARY

The reports of microsporidian parasites from cestodes are cited and discussed. The form *Mucor helminthophthorus* Keferstein, 1861, is considered probably not a protozoon, although subsequent writers have given Keferstein credit for the original description and name of what these writers consider a single species, uncertain as

to genus. By analysis of the several descriptions of these parasites, and by a tabular comparison, the discrepancies are brought out, and it appears that several different forms may have been dealt with. The present species can be assigned to none of these earlier forms, hence is described as new. The specific name "*moniezi*" is used in honor of the first observer who both named and described a microsporidian of cestodes. The present species, because of spore-number variation between one and eight, is placed in the genus *Stempellia* Leger et Hesse, 1910.

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COLORADO TREMATODE STUDIES. I. A FURTHER CONTRIBUTION TO THE LIFE HISTORY OF *CREPIDOSTOMUM FARIONIS* (MÜLLER)*

WILEY W. CRAWFORD

During the summer of 1939 the life history stages of *Crepidostomum farionis* (Müller) were collected from beaver ponds along East River in the vicinity of the Rocky Mountain Biological Laboratory as follows: cercariae in the gills of the clam *Pisidium* sp.; metacercariae encysted in the abdomen of Mayfly naiads; adults in the intestine of the rainbow trout, *Salmo irideus* (Gibbons). Uninfected Mayfly naiads collected from East River were experimentally infected with the cercariae. Fingerling trout, secured from a nearby fish hatchery, when fed both the naturally and experimentally infected Mayfly naiads yielded immature worms upon examination two to four weeks later. Some of these experiments were repeated in the summer of 1940 with similar results. This represents the first experimental work completed on the life history of *C. farionis,* hence forms an additional contribution to our knowledge of the biology of this trematode.

LIFE HISTORY STAGES

Adults

Either sexually immature or adult specimens of *C. farionis* were found in every one of a total of fourteen trout examined between July 2 and August 8 during the summers of 1939 and 1940. The incidence of infection ranged from 2 to 45. The host fish varied in length from 5 to 8 inches and were caught from two beaver ponds at altitudes of 9850 feet and 9950 feet respectively, adjacent to East River, approximately three miles above the townsite of Gothic, Colorado, where the Rocky Mountain Biological Laboratory is located.

The adult trematodes (Fig. 1) conform with the previous descriptions of Nicoll (1909) and Brown (1927), and meet the specific diagnosis for the species as given by Hopkins (1934), with the minor exception of the ratio between the oral and ventral suckers, in which the former is nearer three-fourths of the latter instead of two-thirds. However, there appears to be great variation here, as in some specimens the ventral sucker is nearly twice the size of the oral. In addition, the posterior testis is slightly larger than the anterior. Six sexually mature specimens killed under a cover glass with formalin, alcohol and acetic acid mixture and stained with Ehrlich's haematoxylin ranged from 1.74 to 2.36 mm in length by 0.556 to 0.678 mm in width. This is a somewhat smaller range than that given by Brown (1927) for his specimens.

Ova and Miracida

The operculated ova are elliptical in shape (Fig. 2). Ten living ova which had been standing in water for 24 hours varied in size from 65 to 79 μ in length by 50 to 56 μ in width.

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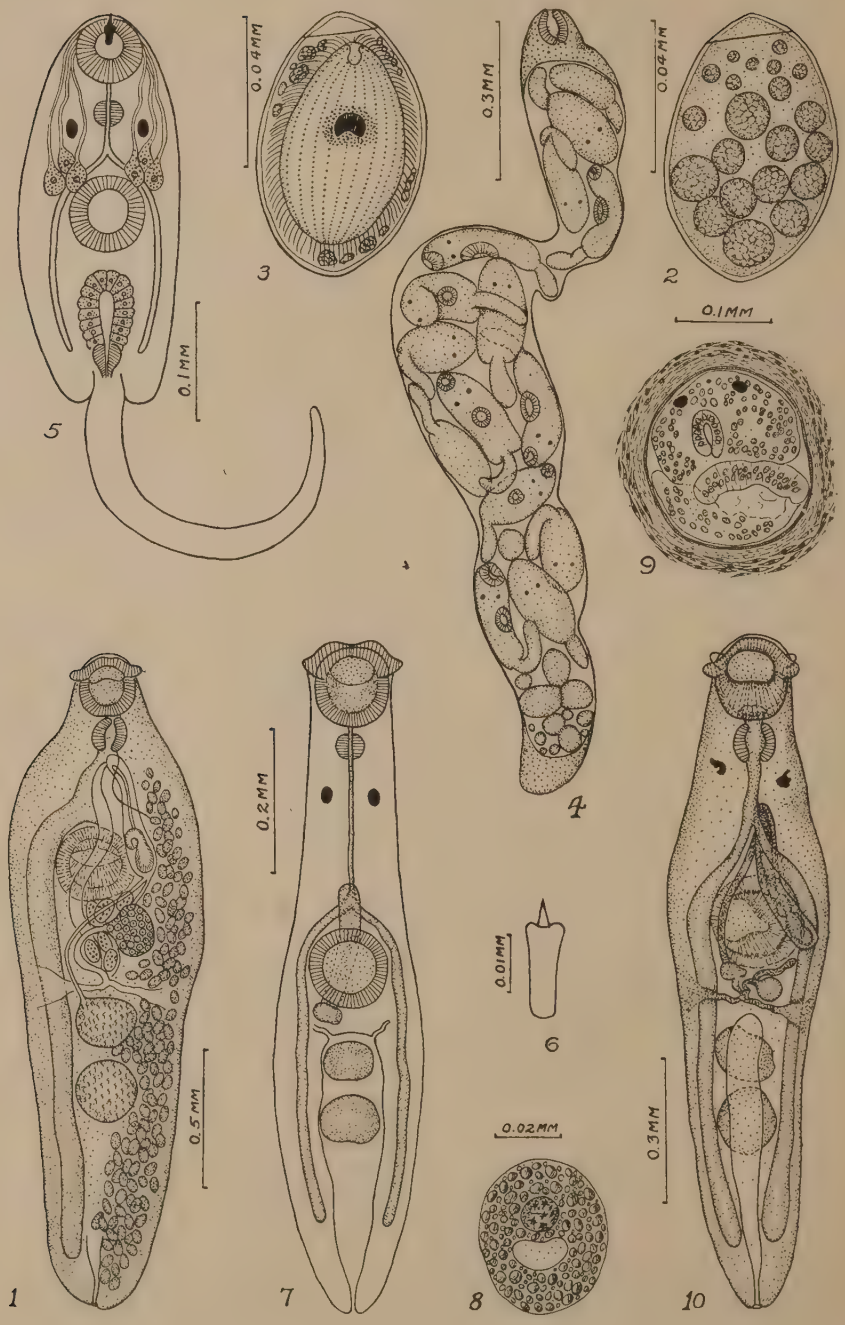
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Repeated observations extended over a period of four weeks failed to disclose any free swimming miracidia. During this time active miracidia were observed within certain ova (Fig. 3), many of which subsequently escaped as evidenced from empty ova. The eyespot appears first as two refractive bodies 8 μ in diameter which later fuse to form a single mass of pigment (Fig. 3). An eyespot measured in a very active miracidium was 26 μ in diameter.

Rediae

The elongated rediae are embedded in the gills of *Pisidium* sp. One redia, filled with cercariae in all stages of development, measured 1.75 mm in length and 0.20 mm at its greatest width (Fig. 4).

Cercariae

Ophthalmoxiphidiocercariae were first described by Brown (1927). Three cercariae killed in hot 10 per cent formalin gave the following average measurements in millimeters: length 0.302, width 0.132, eyespots 0.02 long by 0.019 wide, stylet 0.021 long, oral sucker 0.053, ventral sucker 0.053, prepharynx 0.009 in length and pharynx 0.022 in diameter. These measurements are slightly smaller than those given by Brown (1927), who may have been using living material. The living cercaria studied for Fig. 5 was 0.400 mm long by 0.148 mm wide.

The number of infected clams was not large. Of a total of 178 clams collected between July 5 and 15 from the two beaver ponds, but 5 were shedding cercariae.

Metacercariae

Mayfly naiads, *Ephemera* sp., serve as the transfer host of *C. farionis*. The metacercariae encyst in thin-walled cysts, principally in the fat bodies and musculature of the abdomen. They are oval in shape, rather than pear-shaped as reported by Brown (1927) for his material, and the host cysts become impregnated with a brownish yellow pigment as they mature. Ten of the largest cysts obtained from three different hosts varied in size from 0.247 to 0.296 mm in length by 0.234 to 0.270 mm in width. These figures are slightly larger than those reported by Brown

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Crepidostomum farionis (Müller)

All figures from permanently prepared slides were drawn with the aid of a microprojector. Cytogenous glands were omitted from Figs. 5, 7 and 10 for clarity.

Fig. 1. Adult *C. farionis*. Killed and mounted in somewhat flattened condition, dorsal view; cystogenous glands omitted on left side, intestine on the right side.

Fig. 2. Egg, living specimen.

Fig. 3. Miracidium of *C. farionis*, living specimen, dorsal view.

Fig. 4. Mature redia of *C. farionis* containing cercariae, living specimen.

Fig. 5. Cercaria of *C. farionis*, living specimen, ventral view.

Fig. 6. Stylet of cercaria, living specimen, ventral view.

Fig. 7. Metacercaria of *C. farionis*, living specimen, fully extended condition, ventral view; excretory granules omitted.

Fig. 8. Epithelial cell of excretory bladder of metacercaria (Fig. 7) filled with excretory granules, living specimen.

Fig. 9. Cyst of *C. farionis* in section from the abdomen of a Mayfly naiad killed and sectioned two weeks after laboratory infection.

Fig. 10. Immature adult of *C. farionis*, prepared slide of specimen recovered from trout intestine three weeks after feeding laboratory infected Mayfly naiads, dorsal view.

(1927). Records kept on a total of 32 naiads collected from the two beaver ponds showed 28 of them to be infected. From 1 to 21 cysts were taken from a single host.

The morphology of the mature papillose metacercaria (Fig. 7) agrees in all of the main features with the description of this stage given by Brown (1927) with the exception of the size. He reported a maximum size of 0.65 mm length by 0.24 mm width for his specimens, while it was not unusual to find metacercariae in this material over 0.80 mm in length. While the suckers are approximately equal in size in this material, as well as in his, they are considerably larger.

Observations on the extrusion of excretory granules through the excretory pore when the metacercariae are subjected to pressure under a cover glass revealed that the excretory granules may be intracellular as well as free in the lumen of the excretory bladder. The extruded cells were usually oval-shaped, had a single nucleus and contained a large fluid-filled vesicle in the cytoplasm. One representative cell (Fig. 8) measured 46 by 40 μ; another 40 by 30 μ. The contained granules varied in size up to 4 μ. These cells are probably the lining epithelium of the excretory bladder of the cercaria. After encystment the excretory products are deposited in granular form in the cytoplasm of the epithelial cells. The presence of free granules in the lumen of the excretory bladder could result either from their discharge by the epithelial cells, or by the bursting of the latter after becoming heavily laden with granules.

INFECTION EXPERIMENTS TO DEMONSTRATE THE LIFE CYCLE

Infection of Mayfly Naiads

The Mayfly naiads used in these experiments were collected in swiftly running water either from East River or from some of its tributaries. It was considered safe to use the material from this source as but one cyst of *C. iarionis* was ever found in the scores of such naiads examined.

The infection of the naiads was accomplished by placing from 3 to 5 of them in a small container with a clam from which cercariae were escaping in the evening and leaving them there overnight. It is assumed that the cercariae gained entrance to the body cavity of the naiads by penetrating the exoskeleton of the abdominal wall. Six naiads thus treated were examined in order to determine the extent of the infection. The results were as follows: number one through six revealed 8, 15, 8, 7, 11 and 9 respectively. These cysts, as in the field infected naiads, were located in the abdomen, either in fatty or muscle tissue.

The metacercariae reach full size from within two to three weeks after the time of infection, as shown by the following data: 6 cysts 16 hours after exposure ranged in size from 0.165 to 0.191 mm in length by 0.148 to 0.165 mm in width; 8 cysts 7 days after exposure ranged in size from 0.213 to 0.226 mm in length by 0.200 to 0.217 mm in width; 8 cysts 14 days after exposure ranged in size from 0.243 to 0.261 mm in length by 0.227 to 0.253 mm in width. The cyst wall, which averaged 8.5 μ in thickness in newly formed cysts, increased to 20 μ within two weeks.

Seven days after the penetration of the cercariae the host cyst has not formed as the cysts readily roll free when the abdomen of the infected naiad is torn apart with dissecting needles. The stylet is still present, but has become dislodged. In one instance it was observed to be repeatedly sucked into the mouth and ejected by

the encysted metacercaria. By fourteen days after infection the host wall is well formed (Fig. 9), and the cysts must be freed with the dissecting needles. The yellowish brown pigment so characteristic of the larger field infected cysts has also begun to form.

Infection of Trout

The experiments to establish the trout as the primary host and the relationship between the metacercarial and adult stages of this trematode were carried on in the summers of 1939 and 1940. The trout used averaged about six inches in length and were secured from the State Fish Hatchery at Pitkin, Colorado, 52 miles from the Rocky Mountain Biological Laboratory. They were hatched from eggs secured from Electra Lake, Colorado. Both field infected and laboratory infected Mayfly naiads were fed to these trout, with similar results.

The results are disappointing in that the writer had to leave the Rocky Mountain Laboratory before the yound trematodes had sufficient time to mature in the definitive host. The similarity of structure between both the experimentally reared month-old specimens from field-infected naiads and the three-week-old representatives from labortory-infected naiads and the sexually mature *C. farionis* is indeed striking and leaves little doubt but that all are representatives of one and the same species. (Compare Figs. 1 and 10.)

Experiments Involving the Feeding of Field-infected Mayfly Naiads

During the summer of 1939 four trout were fed with field-infected Mayfly naiads. Autopsy two to three weeks later revealed a heavy infection in each trout. For example, trout number 4 was fed two naiads on July 23, three naiads on July 28 and three naiads on August 8. The autopsy on August 16 showed 57 immature trematodes of three sizes. The smallest specimens averaged approximately 1.0 mm in length; the largest 1.63 mm.

In an attempt to secure sexually mature adult specimens experimentally, feeding experiments were repeated again in the summer of 1940, but without success. For example, host fish number 8, autopsied 4 weeks after feeding 5 naiads, revealed 17 immature specimens, while host fish number 9, autopsied 31 days after feeding 12 naiads, revealed 97 immature specimens.

Experiments Involving the Feeding of Laboratory-infected Mayfly Naiads

These experiments were performed during the summer of 1939. A total of three trout were used and positive results were obtained in each case. Illustrative of the procedure followed and the results secured is the following example: Trout number 3 was fed on July 27 two naiads infected July 15, on August 4 two naiads infected July 19 and on August 11 two naiads infected July 25. The autopsy on August 12 revealed 31 immature specimens of three sizes, 8 of which appeared to have just recently excysted. No sexually mature adult trematodes were found in any of the three trout. All of the young specimens were found in the intestine.

Although the results are not as conclusive as was hoped, since the experimentally fed larval stages did not attain sexual maturity, they do indicate that the cercaria, metacercaria and adult stages occurring in the beaver ponds all belong to the same

species of trematode. Furthermore, since *C. farionis* is the only adult papillose trematode ever found in trout thus far in these waters, it appears reasonable to conclude it is the species under consideration. The results also show that the metacercariae become infective in the second intermediate host from ten days to two weeks after encystment. This is what one would expect since the growing metacercariae reach mature size in Mayfly naiads in about two weeks after infection.

SUMMARY

1. Additional observations and experiments on the life history of *Crepidostomum farionis* (Müller) have been completed.

2. The life history stages occur as follows: adults in the intestine of the rainbow trout, *Salmo irideus* (Gibbons); cercariae in the gills of the clam *Pisidium* sp.; and metacercariae encysted in the abdomen of Mayfly naiads, *Ephemera* sp.

3. Experiments involving laboratory infection of Mayfly naiads with cercariae and the feeding of both field-infected and laboratory-infected naiads to trout are described. This constitutes the first experimental evidence on the life history for this species of trematode.

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A NEW POLYMASTIGINE FLAGELLATE, *COSTIA PYRIFORMIS*, PARASITIC ON TROUT[1]

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One of the most destructive protozoan parasites of trout and other fresh-water fishes is the flagellate *Costia necatrix* (Henneguy), which is common at hatcheries and fish farms in both America and Europe. This parasite was first described by Henneguy in 1887 and has since received considerable attention from protozoologists. In the spring of 1940 an undescribed species, for which the name *Costia pyriformis* is proposed, was found on rainbow trout, *Salmo irideus,* and brook trout, *Salvelinus fontinalis,* at the U. S. Fish and Wildlife Service hatchery, Leetown, West Virginia.

This organism differs greatly in appearance from *C. necatrix* and there is no difficulty in distinguishing between the two species at a glance. The body is distinctly pyriform, rounded anteriorly and tapering to a point posteriorly (Fig. 1), with the greatest diameter about one-third of the distance from the anterior end. On the ventral side there is a spiral groove which starts on the dorsal side at the anterior end and becomes wider and shallower posteriorly. The flagella arise dorsally at the anterior end of the groove.

Ordinarily, there are two pairs of flagella, one shorter than the body, the other somewhat longer, but, frequently, only one flagellum of each size could be distinguished (Figs. 3 and 4). According to Benish,[2] this is also true of *C. necatrix*. The flagella are attached to the blepharoplast, which in the living organism appears as a rounded, clear vesicle on the dorsal side, a short distance from the anterior end. The blepharoplast usually stains intensely with iron-hematoxylin, but, occasionally, only a thin outer layer takes the stain, the central portion remaining colorless.

In addition to the blepharoplast, a number of bright, refringent, rounded or rod-shaped bodies can usually be seen in the living organism. These bodies exhibit an active Brownian movement, indicating the fluid nature of the endoplasm. They stain deeply and uniformly with iron-hematoxylin and other chromatin stains, but their nature has not been determined. They differ from chondriosomes in being highly refractive and resistant to reagents. These chromatoid bodies are usually rod-shaped, but vary greatly in length. Frequently, they are arranged in pairs, end to end, and have the appearance of having been formed from a long rod by a median constriction (Figs. 3 and 4). None were observed, however, which could be interpreted as being in the process of division, but it is significant that the paired rods were frequently shorter than the unpaired. The chromatoid bodies usually number from 10 to 15, but occasionally individuals show a considerably higher or lower number. They have no regular arrangement, but are usually confined to the anterior two-thirds of the body, although they are sometimes found near the posterior end. They are absent, however, from the dorsal side of the body, anterior to the blepharoplast (Fig. 2). This appears to be a specially differentiated mobile region which probably aids in the ingestion of food.

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[1] Published by permission of the Director, Fish and Wildlife Service.

[2] Benish, Johannes 1937 Untersuchungen ueber *Costia necatrix* Leclercq. Z. Fisch. **34**: 755–770.

The nucleus is difficult to distinguish in the living organism, but is easily recognized after staining. It is located near the middle of the body and is of the vesicular type with a rounded deeply staining karyosome in the center. Between the karyosome and the nuclear membrane is a lighter zone containing numerous chromatic granules. A small contractile vacuole (Fig. 3) is located near the nucleus.

Costia pyriformis is considerably smaller than *C. necatrix* with a length of 9–14 microns and a width of 5–8 microns, as compared with a length of 10–20 microns and a width of 5–10 microns for the latter.

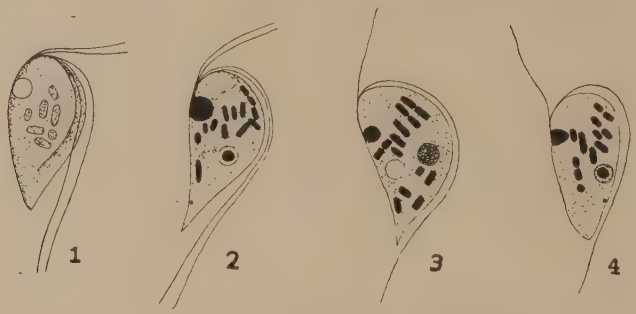

FIG. 1. View of *Costia pyriformis* turned slightly toward the dorsal side to show ventral groove. The blepharoplast appears as a clear rounded vesicle on the dorsal side. Drawn from a specimen killed in osmic vapor, but unstained.

FIGS. 2–4. Side views of *C. pyriformis* stained with iron hematoxylin. The blepharoplast and chromatoid bodies are deeply stained. In Figs. 2 and 4 the nucleus contains a deeply stained karysome. The contractile vacuole is shown in Fig. 3. All figures × 2200.

The habits of the two species are very similar, although *C. pyriformis* seems to show a greater preference for the gills than does *C. necatrix*. However, it also occurs on the body and fins. Like *C. necatrix,* it is closely attached to the epithelium, but when removed to the slide, becomes dislodged and swims about with a characteristic spiral movement which is very different from that of *necatrix*. So far, *C. pyriformis* has been found on trout only, but it is not improbable that, like *C. necatrix,* it may occur on other fishes as well.

ONE SPECIES OF TREMATODE, *NEORENIFER GRANDISPINUS* (CABALLERO, 1938) ATTACKED BY ANOTHER, *MESOCERCARIA MARCIANAE* (LA RUE, 1917)*

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This paper is an account of the invasion by one species of trematode, *Mesocercaria marcianae* (La Rue, 1917) into the body tissues of another trematode, *Neorenifer grandispinus* (Caballero, 1938) n. comb. The attack occurred within an indigo snake, *Drymarchon corias,* from Florida which died in the Lincoln, Nebraska, zoo in February, 1943. Such direct attack of an adult trematode by a larval stage of another is unique so far as the author can determine. Cort (1915) has reported larvae of *Gordius* (Gordiacea) in the parenchyma of eight specimens of a trematode, *Brachycoelium hospitale;* Fischthal (1942) found a *Paragordius* larva in the parenchyma of one of six specimens of a *Plagioporus* species in a freshwater fish; and larvae of *Cotylurus flabelliformis* develop as hyperparasites in the sporocysts or rediae of other trematodes (Cort, Olivier, and Brackett, 1941).

The snake (*Drymarchon*) was infected with approximately 300 *Neorenifer grandispinus* in the esophagus; with a few *Renifer magnus* Byrd and Denton, 1938; with one linguatulid, *Kiricephalus coarctatus* (Diesing); and with one nematode, *Kalicephalus humilis* Caballero, 1938; as well as with *Mesocercaria marcianae.* Several students assisted in collection of these parasites. At the time of collection, when the probable abundance of mesocercariae was not suspected, only one or two of these were noted. Later, three additional specimens were found in vials containing the *N. grandispinus.* They had perhaps become detached after only partially penetrating the latter. Thirteen specimens of *Neorenifer* had been penetrated by mesocercariae.

A brief history of our knowledge of the trematodes involved is necessary. Caballero in a publication dated March–June, 1938, named a trematode *Renifer grandispinus* from *Drymarchon corais melanurus* in Mexico. In October of the same year, Byrd and Denton (1938) described *Neorenifer drymarchon* from *Drymarchon corais couperi,* originally from Texas. There seems to be no important difference in the descriptions of these two species except, perhaps, in egg size. Byrd and Denton report eggs 34 to 36 by 21 μ in size; Caballero reports 41 to 43 by 20 μ. As a rule, small differences in egg size between species should be supported by other differences because of possible variation among individual specimens. Most of the specimens in my collection have egg sizes of 36 to 39 by 19 to 21 μ, but one specimen, otherwise like the others, has somewhat larger, thinner-shelled eggs measuring 41 to 44 by 20 to 24 μ. Therefore, this species seems to have a rather wide variation in egg size, although the eggs within a single specimen are usually very uniform in size. The lengths of body spines are altogether too variable to be considered of specific value. While there might be some question regarding the validity of the genus *Neorenifer* Byrd and Denton, 1938, as distinct from *Renifer,* the genus is tentatively accepted here. Accordingly, the name of the

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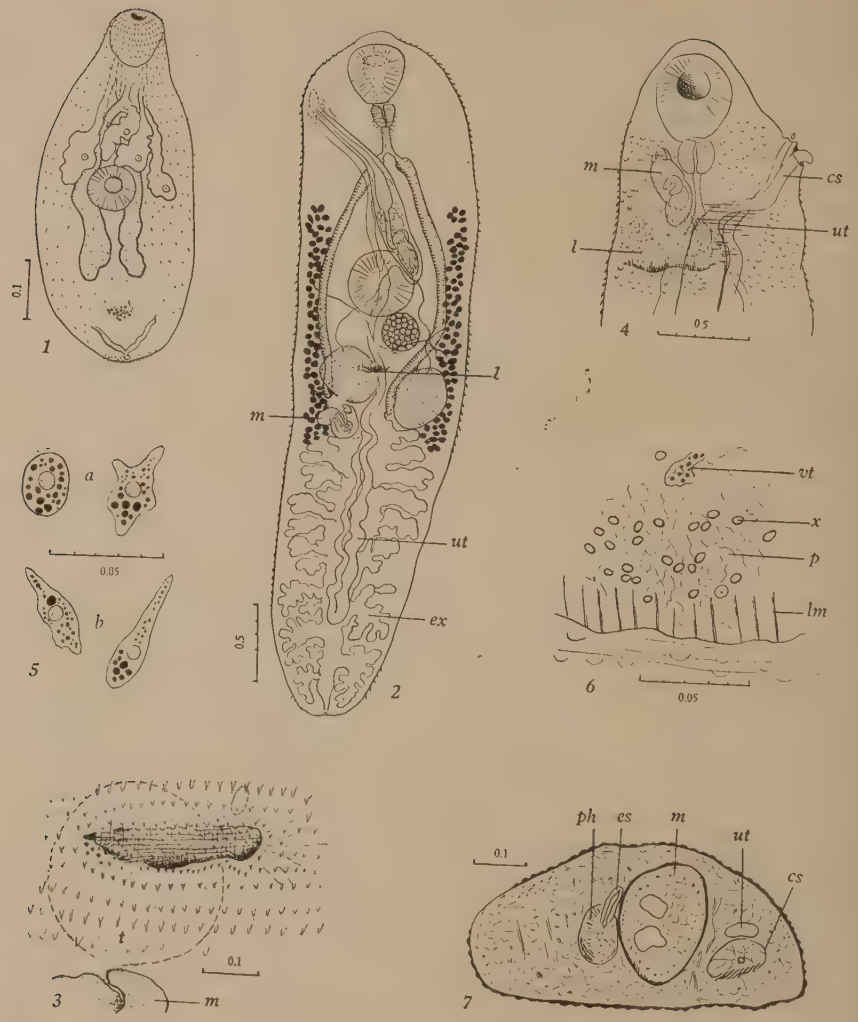

FIG. 1. *Mesocercaria marcianae*. Ventral view. Specimen found in vial with collection of *Neorenifer*.

FIG. 2. *Neorenifer grandispinus*. Dorsal view, showing mesocercaria embedded in parenchyma posterior to left testis, and place of entrance dorsal to testis. The membrane of the testis had been perforated by the mesocercaria, probably at the time of killing.

FIG. 3. Enlarged view of wound caused by entrance of the mesocercaria shown in Fig. 2. In this case, the muscle layers of the body wall have apparently regenerated.

FIG. 4. *Neorenifer grandispinus*. Ventral view of anterior portion showing embedded mesocercaria and lesion produced by its entrance.

FIG. 5. Vitelline cells associated with wounded areas of the parenchyma; (a) two cells from wounded areas; (b) two cells from the parenchyma near yolk glands.

FIG. 6. Enlarged portion of posterior part of wounded area shown in Fig. 4, showing vitelline cell, ovoid bodies on surface of parenchyma, broken ends of longitudinal muscles, and posterior edge of the wound.

trematode is considered to be *Neorenifer grandispinus* (Caballero, 1938) with *N. drymarchon* as a synonym. Caballero's figure (his plate III, Fig. 3) is labeled *"Renifer longispinus"* which must be considered as a second synonym of *Neorenifer grandispinus.*

A morphological feature not noted in the descriptions of this trematode is the branching of the Y-shaped excretory vesicle. The unpaired stem gives off lateral branches which may also be branched (Fig. 2). The main stem forks just posterior to the acetabulum and the crura extend only a short distance anterior to the acetabulum.

In 1917, La Rue described *Cercaria marcianae,* an immature distome, found in large numbers in the tissues (especially in the tail region) of a garter snake, *Thamnophis marciana,* from Texas. Cort (1917) found the same trematode in the tissues of tadpoles and in the digestive tract and body cavity of garter snakes at Douglas Lake, Michigan. He was able to infect garter snakes experimentally but found that the trematode did not develop beyond the stage found in the tadpoles, a stage then called "agamodistome," now, more specifically, a "mesocercaria." The fork-tailed cercaria of this mesocercaria was later described by Cort and Brooks (1928). It is a holostome cercaria developing in *Planorbis* species at Douglas Lake. Bosma (1934) described the life cycle of a related mesocercaria, *M. mustelae,* as requiring four hosts: a snail, a tadpole or frog, a small mammal, and a carnivorous mammal, in which the metacercaria developed into *Alaria mustelae* Bosma, 1931. In 1938, a similar life cycle was described by Olivier and Odlaug for *Alaria intermedia* Olivier and Odlaug, 1938. Cuckler (1940) fed *Mesocercaria marcianae* and *M. intermedia* to rats and mice and found that while some of the trematodes became encysted metacercariae, most of them migrated to the lungs where they became unencysted metacercariae which later reached the trachea, were swallowed and finally reached maturity (*Alaria* spp.) in the intestine. Therefore, only three hosts seem to be necessary although four may be commonly involved. Cuckler's work experimentally completed the life cycle of *Mesocercaria marcianae* to the adult *"Alaria* sp." in a mouse. Probably the adult could also develop in some other mammal eating a mouse containing metacercariae. The discovery of *Mesocercaria marcianae* in a trematode in an indigo snake indicates that this mesocercaria might live temporarily in almost any kind of host.

The indigo snake had been in the Lincoln zoo about a year. So far as is known it had eaten only twice: a mouse several months previously and, about two weeks before its death, a garter snake. Its infection with *Neorenifer* had almost certainly been acquired in Florida since all 300 specimens were fully mature and approximately the same size. The mesocercariae were presumably recently acquired because they

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FIG. 7. Cross-section through a *Neorenifer* infected in pharynx region, showing relative size of mesocercaria, and absence of tissue reaction.

All drawings were made with the aid of a camera lucida.

cs	cirrus sac	p	parenchyma
es	esophagus	ph	pharynx
ex	excretory vesicle	t	testis
l	lesion or wound	ut	uterus
lm	longitudinal muscles	vt	vitelline cell
m	mesocercaria	x	ovoid body of unknown origin

were in the process of migration. Although either the mouse or the garter snake or both could have been the source of the infection, the garter snake seems more probable.

The infection in *Neorenifer* was discovered in one of the last specimens collected while it was being killed under a coverglass. The mesocercaria was squirming about as if in a cyst in the parenchyma. It was completely imprisoned. The killing solution soon penertated its tissues so that only a brief observation of its living condition was possible. Later, 12 additional specimens were found infected while a number of others had been attacked and apparently partially penetrated in one or more places. There is little evidence as to whether the attacks were quite accidental, or selective. The massive numbers of the *Neorenifer* and the small proportion affected suggest the former. But the fact that the mesocercariae should persist in scraping away at a surface armed with such formidable spines as those of *Neorenifer,* and the further fact that one specimen was attacked in five different places, another in three places, suggest either some sort of attraction, or unusual persistence. Thirteen (about 4%) of the 300 specimens contained mesocercariae. Yet 4 (about 30%) of the 13 contained double infections. In 9 cases, the mesocercariae were in the anterior half of the body; in 4 cases in the posterior half. Five infections were close to the pharynx near the central nervous system. In all 4 cases of double infections the two mesocercariae were not far apart.

EFFECTS ON THE MESOCERCARIAE

A total of 17 mesocercariae were observed within the parenchyma of 13 *Neorenifer*. All specimens were completely embedded and unencysted in the parenchyma, sometimes stretched in one direction, often bent at least once. Their size and stage of development corresponded to those of 5 specimens secured outside the trematodes. In general, therefore, no evident effect was seen on the mesocercariae and no advantage to them by the hyperparasitism could be deduced. However, one specimen (in one of the doubly infected *Neorenifer*) was dead. It was almost completely transparent, its outlines and suckers being barely visible; its tissues had disappeared as if by solution; an inflated vesicle-like structure was interpreted as one of the intestinal ceca. There was no evidence as to the cause of the disintegration.

EFFECTS ON *NEORENIFER*

Effects on the host trematode were more evident. Most of them, however, were limited to the surface area of penetration. In almost all cases, the place of penetration could be detected not very far from the body of the mesocercaria. In one of the double infections, one larva had entered the ventral surface just anterior to the acetabulum and ploughed backward dorsal to the left border of the acetabulum until its anterior end was on a level with the posterior edge of the acetabulum (a distance of 0.645 mm). Here it met (being almost in contact with) the anterior end of another mesocercaria which had penetrated the dorsal surface opposite the oötype just posterior to the opening of Laurer's canal and traveled to the left about 0.36 mm. Strange rendezvous!

Once embedded in the parenchyma the mesocercariae seemed to stimulate little or no reaction except in two cases where severe damage was done to reproductive organs. Even in the case of one specimen implanted directly in or on the central nervous system near the posterior border of the oral sucker, no injury was evident.

Two specimens were effectively castrated by the mesocercariae, in one case both egg and sperm production being suppressed. Probably the most easily damaged organs of a trematode are the vitelline glands. In one specimen, a mesocercaria had penetrated longitudinally through the middle of one of the vitelline fields. Excessive vitelline secretion had flooded the entire length of the uterus so filling it that only a few highly abnormal, abortive eggs occurred. The condition was similar to that described by Manter (1927) for *Neorenifer validus* (Nicoll). In the same specimen, pressure on the vasa efferentia either by the mesocercaria or by the swollen, yolk-filled uterus seems to have closed these vessels to the flow of sperm cells. At least, the vasa efferentia were well filled with sperm cells posterior to the level of the mesocercaria (near the base of the cirrus sac) but were empty anterior to that level. The seminal vesicle was also completely empty of sperm cells. No eggs or sperm cells could pass from this specimen, although both the ovary and the testes were normal.

The other castration was evidently caused by the mechanical destruction of both testes. The head of the mesocercaria was embedded within the smashed remains of one testis and was surrounded by scattered testicular material. The other testis had been completely destroyed with very few remains of sex cells discernible, but the anterior end of the testicular membrane with the vas efferens attached could be seen. This specimen still had the seminal vesicle filled with sperm cells but its power to function as a male could have continued but a short time.

Most common effects of the parasitism were on the body surface of *Neorenifer*. At the place of attack the cuticula including its spines was scraped or digested away, sometimes over a considerable area. The circular, longitudinal, and diagonal muscles were exposed along the edges of the trauma and usually the parenchyma was exposed in the central area. Such an affected area could almost always be seen not far from the embedded mesocercaria and, in addition, similar abrasions were not uncommon on uninfected specimens indicating unsuccessful, perhaps interrupted, attacks. Considering the extent of the wounds (sometimes almost across the body), there was remarkably little reaction by the tissues. The exposed parenchyma was slightly more granular, with some slight concentration of nuclei, perhaps because of shrinkage of vesicular cells near the surface. The stroma of the parenchyma became more closely fibrous at the surface and a thin membrane-like layer was apparently formed by the cell membranes of parenchyma cells. Frequently, a light brownish color was present in the affected area, and scattered through the parenchyma were a number of small, ovoid particles of light brown color and uniform texture (Fig. 6). The origin and nature of these particles could not be determined. It is thought they were either dead nuclei of parenchyma cells (which they resemble in size) or coalesced particles of shell-like material liberated by yolk cells which had migrated to the spot. It seemed evident that individual cells from yolk glands did migrate to the wound since these cells in amoeboid shape could be found in the parenchyma near the yolk glands and in the affected area (Fig. 5). In the younger wounds they were seen in moderate number but were sometimes apparently lacking especially in the older wounds.

There was no evidence of bacterial infection.

In what seemed to be older wounds, there were continuous layers of longitudinal and circular muscles although mesocercariae had evidently entered at those spots. Therefore, it seems probable that the muscle layers had regenerated. Regeneration

of cuticula was slower and must have occurred, if at all, from the edges of the wound. The small size of the opening of some wounds indicated some such regeneration.

SUMMARY

A specimen of an indigo snake, *Drymarchon corais,* from Florida which died in the Lincoln, Nebraska zoo, contained among other parasites about 300 specimens of *Neorenifer grandispinus.* Specimens of *Mesocercaria marcianae* were found embedded and unencysted in the parenchyma of 13 of the *Neorenifer.* The mesocercariae had invaded the other trematode by direct penetration of its cuticula and body wall. Considerable damage was done to the surface area involved in the penetration, with loss of cuticula and muscles of the body wall, leaving the parenchyma exposed. The exposed stroma of the parenchyma seemed to form a fibrous protection while the deeper layers were unaffected; there was a slight accumulation of nuclei and often a deposition of brownish, somewhat refractile bodies of unknown origin; and there was some migration of yolk cells to affected areas. Regeneration of muscle fibers seemed to occur, as well as a slow addition of cuticula to the edges of the wounds. The only internal damage was to the reproductive organs of two specimens. In both cases, the male system had been put out of commission; once by the mechanical constriction of the vas efferentia, in the other case by the destruction of both testes by direct attack of the mesocercaria. One mesocercaria was embedded near the vitellaria and a surplus of yolk material had flooded the uterus and suppressed all normal egg production.

Neorenifer grandispinus (Caballero, 1938) is named as a new combination with the following synonyms: *Renifer grandispinus* Caballero, 1938; *Neorenifer drymarchon* Byrd and Denton, 1938; *Renifer longispinus* Caballero, 1938.

Specimens showing the features described in this paper are deposited in the U. S. Nat. Mus. Helm. Coll., No. 36886.

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STUDIES ON THE BIOLOGY OF THE ARGASID TICK, *ORNITHODOROS NICOLLEI* MOOSER[1]

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Distribution: Mexico: States of Guerrero, Puebla, Colima, and Jalisco (Mooser, 1932; Brumpt, Mazzotti and Brumpt, 1939; Mazzotti, 1942).

Hosts–Habitats: Neotoma, man, and dogs; also found on a rattlesnake in the Zoo in St. Louis, Missouri. Native houses in Mexico.

Feeding time: These ticks feed readily on mice and guinea pigs. Larvae may engorge and detach voluntarily in less than 15 minutes or they may remain attached for several days before completing engorgement. Nymphs and adults completed engorgement in from 15 to 23 minutes.

Molting: June 8 to 11, 1942 eighty-five larvae[2] completed engorgement on a guinea pig. They were placed separately in shell vials, numbered serially, and accurate records made until all surviving ticks reached the adult stage. This requires individual feeding in all nymphal stages. The ticks were stored at room temperature in humidity jars containing a saturated solution of ammonium chloride.

All larvae molted in 9 to 13 days; first nymphs in 8 to 11; second nymphs in 6 to 20; third nymphs in 7 to 14; fourth nymphs in 11 to 39; fifth nymphs in 12 to 48, and 3 sixth nymphs in 21 days. The spread in molting time was greatest in the fourth and fifth nymphal stages and perhaps more nearly approaches conditions in nature as these ticks detach easily when disturbed and the degree of engorgement influences molting time.

Nymphal stages: As in several other species of *Ornithodoros,* the period of the greatest mortality was during the larval molt. In this series 18 ticks died at the time of the larval molt and three at the first nymphal molt. Sixty-four were reared to adults (37 ♂♂, 27 ♀♀). The first adult, a male, appeared 70 days following the larval feeding and the last, a female, at 160 days. Following the fourth nymphal molt there were 31 ♂♂ and 2 ♀♀; at the fifth, 6 additional males and 22 females, and at the sixth, 3 females.

Oviposition: Observations were made on 21 females, 12 of which were reared and 9 as received from Mexico. Data on the preoviposition period—time from the last engorgement to the appearance of the first egg—indicate a definite trend toward a resting period during the summer months. This is in marked contrast to certain northern species which show a similar period of inactivity during the winter months. As a rule, females that engorged in late autumn began egg-laying in a much shorter time than females engorged in the spring. Of 16 females engorged in November, 11 began oviposition in 15 days or less; the shortest interval was 7 days. On the other hand, 3 females engorged, respectively, in April, May, and July began to oviposit in November, November, and December. The periods were accurately observed in 36 instances; these varied from 7 to 233 days.

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[1] From the Rocky Mountain Laboratory (Hamilton, Montana) of the Division of Infectious Diseases, National Institute of Health.

[2] The stock from which these ticks were reared was received from Dr. Luis Mazzotti, Instituto de Salubridad y Enfermedades Tropicales, Mexico, D. F.

Number of eggs: Forty-two counts were made on 21 females, with from one to 4 counts for each female. The minimum number of eggs was 184 and the maximum 631. Seventy per cent were above 300, 33 per cent above 400, and 20 per cent above 500. There is also a tendency in this species to oviposit a second time without further feeding. The counts for 10 females ovipositing twice, November and March, were 416 - 301, 251 - 184, 591 - 217, 492 268, 563 - 184, 609 - 288, 296 - 315, 608 - 309, 238 - 335, and 382 - 233. As a rule, the number of eggs increases from the first to at least the third or fourth oviposition. As representative of such increases, the number of eggs at the first and third ovipositions, respectively, for 7 females was as follows: 251 – 316; 326 – 476; 344 – 609; 286 – 563; 459 – 631; 266 – 540; 314 – 591.

Hatching time: Thirty-three observations were made. The period between the appearance of the first egg and the first larva varied from 17 to 36 days with less than 25 days in 70 per cent of the cases.

Fertility: The ratio of larvae to eggs is relatively high as compared to the results obtained in some other species of *Ornithodoros* and may not decrease perceptibly, without further mating, during at least 4 ovipositions over a period of 8 months. Females that had no opportunity to mate after early April produced eggs at the third oviposition in late December with 98 per cent hatching. Two females engorged April 4, 1942 and not subsequently exposed to males, failed to oviposit until April 1943. There were 444 and 197 eggs, respectively, with 98 per cent fertility in each case.

Relation to certain infectious diseases: Naturally infected ticks have not been reported. Experimentally, the transmission of the rickettsiae of the spotted fevers of the United States of America, Colombia, and Brazil and transmission through the egg to the first generation have been accomplished (Davis, in press). *Rickettsia diaporica* (American Q fever) is not transmitted by tick feeding but is conserved in the tissues of the tick, as demonstrated by injection, for 223 days and probably throughout the life of the tick. *Pasteurella tularensis* is not transmitted by tick bite but is also conserved in the tissues for at least 207 days, as shown by injection. This organism is definitely harmful to the ticks; molting is delayed and the infected ticks become dwarfed.

SUMMARY

The argasid tick *Ornithodoros nicollei* found in the states of Guerrero, Puebla, Colima and Jalisco, Mexico, feeds, naturally, on *Neotoma*, man, and dogs. It also feeds readily on laboratory animals. Feeding is rapid with the exception of *some* larvae which may require several days to complete engorgement. Males mature first. There are from 4 to 6 nymphal stages. Based on 42 counts, the number of eggs varied from 184 to 631. The number of eggs increases from the first to the third or fourth oviposition. There is a tendency to oviposit a second time without further feeding. Fertility was as high as 98 per cent in females that had not mated for at least one year.

Ticks of this species experimentally transmit the rickettsiae of the spotted fevers of the United States of America, Colombia and Brazil with marked facility. Transmission through the egg was demonstrated in Brazilian and Colombian spotted fever. *Rickettsia diaporica* and *Pasteurella tularensis* are not transmitted by bite but are conserved for long periods in the tissues of the tick as demonstrated by injection.

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FURTHER ATTEMPTS TO TRANSMIT *PASTEURELLA TULARENSIS* BY THE BEDBUG (*CIMEX LECTULARIUS*)*

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Continued unqualified statements in current literature that the bedbug (*Cimex lectularius*) is a *vector* of *Pasteurella tularensis* suggested the desirability of a method of feeding that would eliminate the possibility of fecal contamination of the host.

In studies reported by Francis and Lake (1922) and by Francis (1927) the infection of mice by bugs was effected only by interrupted feeding of the bug during the infective feeding or subsequently when the mouse tails became covered with bug feces during the biting experiment which lasted an hour. It was also determined that bugs remain infected throughout their lives. Tests for transmission through the egg were not made but microscopic examinations failed to reveal organisms in the reproductive organs. Bogenko (1935) reported infection by bite 15 hours after the infective feeding, and Kamil and Bilal (1938) also have reported transmission by *bite*. Bilal (1941) further reported transovarial transmission.

Methods: The bugs were placed under a feeding capsule attached to a guinea pig. The characteristic feeding stance of the bug is in a vertical position about the periphery of the enclosed space. Immediately after engorgement, which rarely required more than 5 minutes, the bugs migrated to the top of the capsule and were removed. Following each feeding they were placed in a clean shell vial containing a fresh strip of blotting paper.

Experiment 1: Oct. 30, 1942, forty bugs in the second nymphal stage engorged on a guinea pig ill with tularaemia. Immediately after the infective feeding, 5 of them were ground in saline and injected subcutaneously into a fresh guinea pig, resulting in death from tularaemia the third day.

The remaining bugs, in diminishing numbers, were given 9 test feedings over a period of 4 months with no evidence of infection in any of the 9 host guinea pigs.

Two bugs that were tested by injection following the fourth test feeding caused death of the guinea pig on the fourth day. One, 1, 2, 1, 7 and 14 bedbugs respectively, died following the fourth to ninth feeding. One hundred twenty-five days following the infective feeding the 8 remaining bugs were ground separately in saline and injected into guinea pigs. There was no evidence of infection over a period of 10 days.

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* Contribution from the Rocky Mountain Laboratory (Hamilton, Montana) of the Division of Infectious Diseases of the National Institute of Health.

It must be assumed that these 8 bugs did not acquire *P. tularensis* at the time of the infective feeding or that they subsequently lost the organism. In either case it appears likely that bugs that do not harbor the organism outlive infected bugs. Infected bugs were present through at least 7 test feedings as shown by the injection of washings of the strips of blotting paper on which the bugs rested.

Transovarial transmission: The possibility of infection through the egg was tested by feeding and by subsequent injection of the progeny of the bugs following 5 successive ovipositions. The number of progeny tested was 26, 130, 143, 85, and 96 (ovipositions 1–5 following fourth to eighth feedings). Using the feeding methods outlined above, these bugs were given three test feedings and were injected directly after the last feeding. None of the 15 host guinea pigs or the 5 injected guinea pigs became infected. It seems reasonable to believe some of these were the progeny of infected bugs or, if not, that infection inhibits oviposition.

Infectivity of feces: The infectivity of the feces was tested by the injection at varying intervals of saline washings of the strip of blotting paper.

Thirteen such injections were made. Injections made 10, 28, 111, 119 and 130 days following the infective feeding and 10, 10, 21, 8 and 19 days, respectively, following the last previous feeding were non-infective. Injections made 36, 37, 39, 47, 48, 49, 72 and 88 days following the infective feeding and at intervals varying from one to 16 days following the last previous feeding proved fatal to the injected guinea pigs.

SUMMARY AND CONCLUSIONS

1. Bedbugs (*Cimex lectularius*) failed to transmit *P. tularensis* when a "clean" method of feeding was used, eliminating fecal contamination of the host.

2. Progeny of these bugs resulting from five successive ovipositions failed to infect guinea pigs at three test feedings and by subsequent injection.

3. Bug excrement is infective. *Pasteurella tularensis* seems to shorten the life of the bug.

4. Although infection may take place by fecal contamination, transmission is not effected by bite other than by mechanical transfer during interrupted feeding. Transovarial transmission reported by Bilal has not been confirmed.

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NOTES ON THE LUNGWORMS (GENUS *PROTOSTRONGYLUS*) OF VARYING HARES (*LEPUS AMERICANUS*) IN EASTERN NORTH AMERICA*

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Numerous observations on lungworms in wild hares (*Lepus timidus, L. europaeus*) have been made at various places in Europe during the past century and a half, but it has been only within the last ten years that parasitological examinations of Varying hares (*Lepus americanus*) have revealed the presence of related metastrongyloids in this hemisphere. During the past four years one of us (Goble) has had the opportunity of autopsying a number of Varying hares from the eastern United States and Canada. It is the purpose of this paper to record observations on the incidence and identity of the lungworms in these animals.

Boughton (1932) found lungworms in 25 of 30 (83 per cent) Varying hares in southern Manitoba. He described these nematodes and named them *Synthetocaulus leporis*. Green and Shillinger (1935a) reported lungworms in 6 of 75 (8 per cent) hares (*L. americanus phaeonotus*) in Minnesota. This survey was conducted by J. F. Bell during the months of April through July inclusive, and the worms were designated as *Synthetocaulus leporis*. In collections made in Minnesota and Wisconsin during October and November of the same year Green and Shillinger (1935b) noted lung lesions in 25 of 40 (62 per cent) hares. These lesions were described and attributed to the presence of lungworms which were called *Synthelocaulus cuniculi*. MacLulich (1937) stated that lungworms in hares (*L. a. americanus*) in Ontario "were only occasionally found and then in small numbers."

Of the animals which were examined in the course of the present study 87 were *Lepus americanus virginianus* from New York and 44 were *L. a. struthopus* from New Brunswick. They were collected during the months of November through March inclusive. The New York specimens came from three different localities in the Adirondack region.

INCIDENCE

Examination of the lungs of 87 hares from New York state revealed the presence of lungworms in 42 per cent of the males (21 of 50) and in 40 per cent of the females (15 of 37). It seems permissible, therefore, to combine the records for the two sexes in determining the incidence of these parasites.

The incidence in the hares from the township of Long Lake in the central Adirondacks was 33 per cent (12 of 36), while that in Dickinson township on the northern edge of the mountains was 48 per cent (16 of 33). In Colton township, which lies between these, 44 per cent (8 of 18) were infected.

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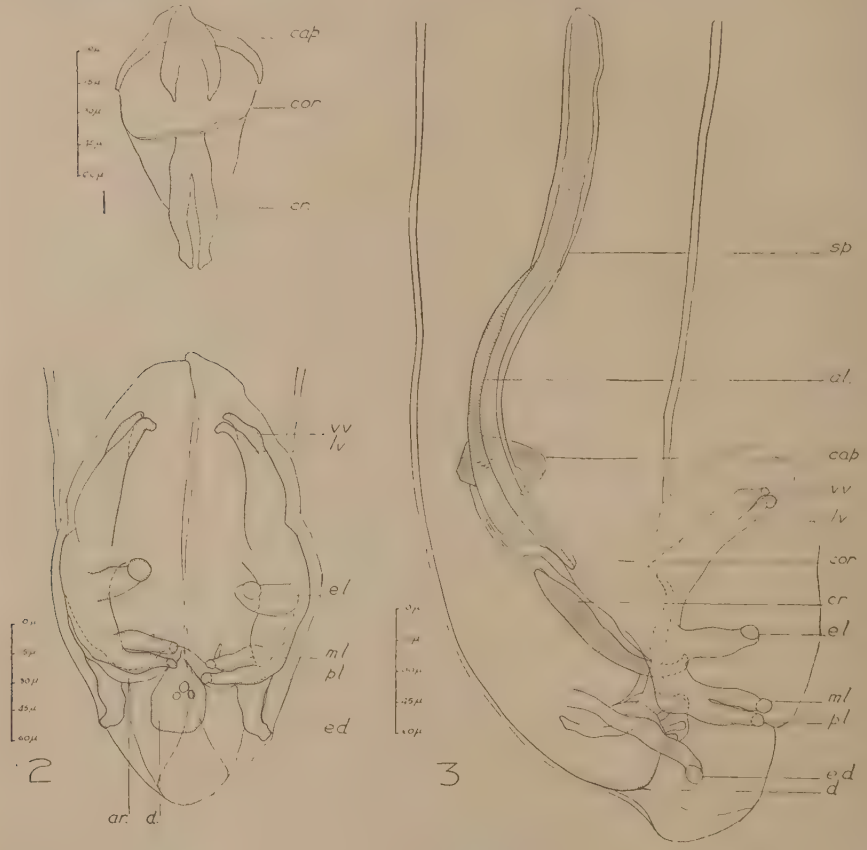
* We wish to acknowledge our appreciation of the helpful criticism and kind coöperation of Dr. Gerard Dikmans, U. S. Bureau of Animal Industry, Washington, D. C.

Only 11 per cent (5 of 44) of the hares from New Brunswick were infected.

The number of specimens examined did not warrant analysis on a yearly or monthly basis.



Protostrongylus boughtoni nom. n.

FIG. 1. Gubernaculum of the male: *cap.*, capitulum, *cor.*, corpus, *cr.*, crura.

FIG. 2. Posterior end of male, ventral view with spicules and gubernaculum omitted: *ar.*, sclerotized arc, *d.*, dorsal ray, *e.d.*, externodorsal ray, *e.l.*, externolateral ray, *l.v.*, lateroventral ray, *m.l.*, mediolateral ray, *p.l.*, posterolateral ray, *v.v.*, ventroventral ray.

FIG. 3. Posterior end of male with spicules and gubernaculum: *al.*, spicular ala, *sp.*, spicule.

FIGS. 1–3 made with the aid of the camera lucida.

IDENTITY

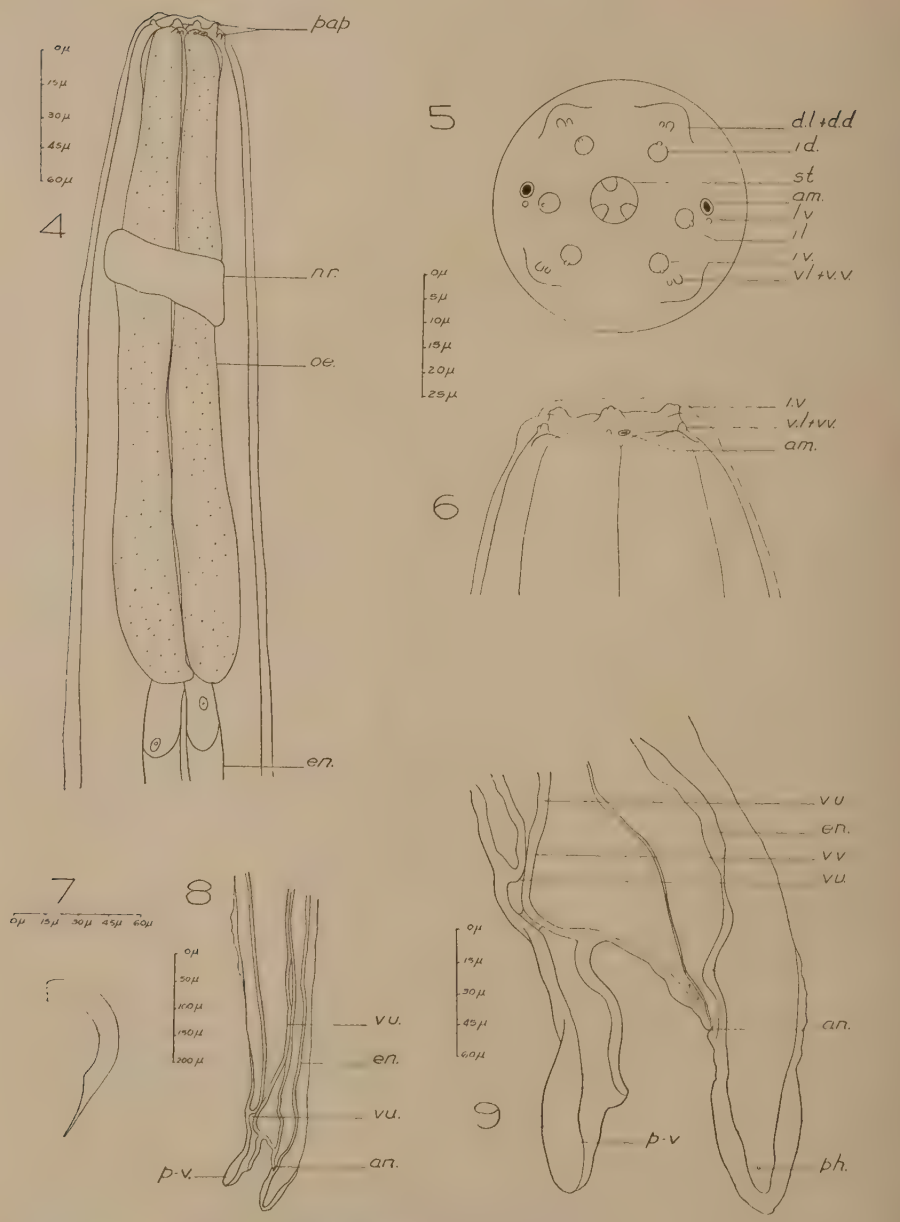
Examination of numerous specimens collected from hares taken in New York, New Brunswick, and Minnesota revealed that these lungworms were morphologically identical with *Synthetocaulus leporis* Boughton, 1932. Dr. Gerard Dikmans of the U. S. Bureau of Animal Industry has examined lungworms from hares from Michigan and Massachusetts and has concluded that they too are probably the same species.

Kamenskii (1905) discussed the identity of the lungworms of European hares and proposed the genus *Protostrongylus* to include the hare lungworms studied by his student Nikulin and related forms described by other authors from ruminants as well as hares. The genus *Protostrongylus* preceded by two years the genus *Synthetocaulus* Railliet and Henry, 1907, which was erected to include the same group of species. *Protostrongylus* has been accepted by most workers since its priority over *Synthetocaulus* was pointed out by Leiper (1926). Russian writers since 1933, however, have reverted to the use of the latter generic name, which had been in general usage from the time it was proposed by Railliet and Henry (1907) to the time *Protostrongylus* was reëstablished by Leiper. Kamenskii (1905) did not select a type species for *Protostrongylus*, but Cameron (1927) designated *P. rufescens* (Leuckart, 1865) Kamenskii, 1905, as genotype. Hence this genus appears to be validly constituted, and reversion to *Synthetocaulus* unjustified.

The transfer of the species *S. leporis* Boughton, 1932, from *Synthetocaulus* to *Protostrongylus* would seem, therefore, to be in order if it were not for the fact that Kamenskii proposed the name *"Protostrongylus leporis* sp. inquirenda (an dubia?)" for *Filaria pulmonalis* Frölich, 1802. The specific appellation *cuniculi* inadvertently used by Green and Shillinger (1935b) must therefore come into consideration as a possible name for the species. It appears to us that the acceptance or rejection of this name cannot be definitely determined by application of the Rules of Nomenclature, which are here subject to conflicting interpretation. Article 25 of the Rules states that a specific name shall have a status of availability if a "definition accompanies it by which the species named can be distinguished from other species." The present case resolves upon a subjective opinion as to whether information given by Green and Shillinger constitutes an adequate definition. In all probability forms referred to by them can be identified as Boughton's species. However, other factors contraindicate the use of *cuniculi*, although these are not specifically covered by the Rules. They are: 1) the medium of publication of Green and Shillinger's article was a mimeographed monthly report with a limited distribution and therefore widely unavailable, especially to foreign investigators; 2) the use of *cuniculi* was inadverent, *leporis* doubtless having been intended; 3) the appellation *cuniculi* was thus not designated as a new name for Boughton's species and was not intended to replace *leporis*. Therefore we reject *cuniculi* as a specific designation for Boughton's species. However, in case difference of opinion should arise among future investigators, we are including for the benefit of the large number to whom Green and Shillinger's account will be unavailable, a direct quotation in the discussion which follows the revised diagnosis of the species under consideration.

In a list of the nematode parasites of the Varying hare in Ontario, MacLulich (1937) gave *"Protostrongylus leporis.* In duodenum . . ."; farther down, *"Synthetocaulus leporis.* Lungworms . . ." appeared. It is apparent that the first of these was either a misidentification or an error in the indication of habitat. In all probability both names refer to the same species of hare lungworm.

In view of the preoccupation of *Protostrongylus leporis* by Kamenskii's species inquirenda, we propose the name *Protostrongylus boughtoni* nom. n., to replace *Synthetocaulus leporis* Boughton, 1932. It seems desirable to refigure the posterior end of both sexes and to make certain other additions to the description of the worm. In the following description our measurements, based on 12 male and 16 female worms, are followed parenthetically by Boughton's.



Protostrongylus boughtoni nom. n.

FIG. 4. Anterior end and esophagus: *en.*, enteron, *n.r.*, nerve ring, *oe.*, esophagus, *pap.*, cephalic papillae.

FIG. 5. Anterior end, en face view: *am.*, amphid, *d.l.* + *d.d.*, dorsolateral + dorsodorsal papillae, *i.d.*, interodorsal papilla (on lip), *i.l.*, interolateral papilla, *i.v.*, interoventral papilla, *l.v.*, laterovéntral papilla, *v.l.* + *v.v.*, ventrolateral + ventroventral papillae, *st.*, stoma.

Protostrongylus boughtoni nom. n.

(Figs. 1–9)

Synonymy: Synthetocaulus leporis Boughton, 1932; ? *Synthelocaulus cuniculi* Green and Shillinger, 1935; ? *Protostrongulus leporis* (Boughton, 1932) MacLulich, 1937 (nec *Protostrongylus leporis* Kamenskiĭ, 1905).

Description: Anterior end of both sexes with six small lips bearing the internal circle of papillae. External circle of ten papillae, dorsodorsals and ventroventrals reduced in size. (Figs. 4–6.)

Male: Length 13–26 mm (23–28), width 160–250 μ (154–176), esophagus 270–360 μ (276–330), spicules 260–320 μ (260–305), capitulum 50–55 μ (about 60, from drawing), corpus 30–45 μ (weakly sclerotized), crura 70–92 μ (68–84). Appearance of bursa, sclerotized arcs, and gubernacular complex shown in Figs. 2 and 3; gubernaculum illustrated in Fig. 1.

Female: Length 21–36 mm (25–40), width 200–300 μ (154–242), esophagus 325–400 μ (not given), vagina 2–2.4 mm (1.9–2.4), vulva to tip of tail 160–200 μ (135–194), anus to tip of tail 100–110 μ (about 90, from drawing), provagina 100–154 μ (90–110), eggs 50–70 × 40–60 μ (65–75 × 40–50). Body width narrows abruptly from about 125 μ anterior to vulva, to about 65 μ posterior to it. Phasmids present as tiny papillae near tip of tail. Appearance of provagina and adjacent structures shown in Figs. 8 and 9.

Larva: First stage larvae found in lungs and feces are 320–360 μ long and 14–16 μ wide. They have relatively long, straight caudal appendages that differ from S-shaped types reported for several other species of group. Tail pictured in Fig. 7.

Hosts: Varying hare, or snowshoe rabbit, *Lepus americanus* Erxleben.—4 subspecies: *L. a. americanus, virginianus, struthopus, phaeonotus.*

Geographic distribution: Manitoba, Minnesota, Wisconsin, Michigan, Ontario, New York, Massachusetts, New Brunswick.

Protostrongylus boughtoni nom. n. must be distinguished from the other species of the genus with a prominent provagina. It differs from *P. stilesi* Dikmans, 1931, and *P. raillieti* (Shul'ts, Orlov, and Kutas, 1933) Cameron, 1934, in the shape of the distal portions of the crura; from *P. kamenskyi* Shul'ts, 1930, in the length of the spicules and shape of the capitulum; from *P. austriacus* Gebauer, 1931, in length of the spicules and of the vagina; and from *P. coburni* Dikmans, 1935, in the structure of the capitulum and of the externodorsal ray. The latter in *P. coburni* is unique in the possession of a lateral nob or branch at about the middle of its length.

The gubernaculum of *P. boughtoni* is particularly noteworthy in that both corpus and crura appear to have lateral cuticular expansions as illustrated in Fig.1.

Further reference should be made to the species found in European hares. A careful analysis of the literature on these forms, particularly of the paper by Kamenskiĭ (1905), convinces us that there are at least two species occurring in Europe. Both were considered by Kamenskiĭ, but confused with certain forms in ruminants. A complete analysis of the situation is not within the scope of this paper, but is involved in the complex and confused systematics of the entire genus *Protostrongylus*. Apparently Kamenskiĭ dealt with *P. kamenskyi* Shul'ts, 1930, and with a form called by Kamenskiĭ *P. terminalis* (Passerini, 1884) and by Cameron (1927) *P. commutatus* (Diesing, 1851). There is strong evidence to indicate that the latter species is identical with *Filaria pulmonalis* Frölich, 1802, and we believe that it should therefore be designated *Protostrongylus pulmonalis* (Frölich, 1802) comb. n.

Fig. 6. Anterior end, lateral view showing cephalic papillae.

Fig. 7. Posterior end of larva.

Fig. 8. Posterior end of female (low power): *an.*, anus, *p-v.*, provagina, *vu.*, vulva, *v.u.*, vagina uterina.

Fig. 9. Posterior end of female (high power): *ph.*, phasmid, *v.v.*, vagina vera.

Figs. 4–9 made with the aid of the camera lucida.

P. boughtoni is distinct from both *P. kamenskyi* and *P. pulmonalis*. An extended analysis of the problems in the systematics of hare and ruminant lungworms of the *Protostrongylus* group is now in preparation.

The following is a direct quotation of Green and Shillinger's (1935b, pp. 91–95) discussion of the lungworms and conditions of lungworm infection in the hares studied by them:

"From all hares showing gross pathology of the respiratory tract, sections were taken for microscopic examination. The gross lesions noted varied to some extent but on the whole conformed to a picture typical of lungworm (*Synthelocaulus* [sic] *canicali*) infestation. The most characteristic lesions are easily recognized. There are usually a number of small, hard, discrete, granular areas which can be palpated. They are yellowish gray in color and there seems to be a tendency toward necrosis in the surrounding lung parenchyma. These lesions may be spread indiscriminately over any part of the lung but occur in groups rather than in single foci. When the foci are cut across with a knife, a clear, viscid exudate may be forced from the bronchi by the exertion of slight pressure. The exudate is gelatinous in consistency. If a portion of the lung containing such lesions is macerated in water and floated, it is possible to demonstrate the presence of lungworms in most instances. Pleural adhesions were encountered in two of the hares. In three of the animals showing lungworm foci, the spleen was enlarged to a slight extent. Small abscesses or cysts were noted in the liver in a few cases. Definite caseous necrosis was apparent in the lungs of two hares for which a diagnosis of tularemia was subsequently established either by microscopic examination or by isolation of *Pasteurella tularensis*.

"Microscopic examination of lung from 21 of the 25 hares revealed numerous departures from the normal. In almost every hare there was proliferation of macrophages into the alveoli with varying amounts of pigmentation. A bronchitis and a peri-bronchitis were found in all except two hares. These conditions were characterized by an intense degeneration and subsequent desquamation of the epithelial cells. In many cases the lumen of the bronchi was plugged. The exudate in the bronchi was composed of several elements: erythrocytes, macrophages, epithelial cells, fibrin, and serum. In a few cases the bronchial epithelium showed a preliminary hyperplasia. A waxy substance was also found in some bronchi. A foreign body reaction was associated with the bronchial findings in a few instances. Lymphocytic infiltration was found in only two hares, and in one of these there was an associated necrosis. Cuffing of the vessels was to be seen in four animals. Two types of lungworm infestation occurred. One type of worm was a small organism composed of minute cells containing basophilic nuclei. This type had a definite chitinous sheath. It occurred primarily in the alveoli. The other worm was located in the bronchi and was a typical adult lungworm. The latter worm was a large organism showing the development of various tissues and organs.

"Among the findings in the lungs examined were a number of bodies not previously seen. There were three distinct types of body which showed a tendency toward gradation into each other. The first type appeared a multicellular body and bore a marked resemblance to histoplasma, although staining reactions were not typical. Usually the body consisted of fewer than 5 or 6 cells. In these multinucleate bodies the cell boundaries were indistinct and the cells were joined in such a manner that the periphery was scalloped. The cytoplasm was acidophilic and

filled with acidophilic droplets. The nuclei were situated near the periphery of the cells. They were subject to division as shown by the mitotic figures. Structures of this type varied somewhat as to number of cells, but each cell resembled every other one morphologically. There were also a number of similar single cells about 60–70 [μ] in diameter and definitely round, free in the alveoli of many of the hares. The cytoplasm was acidophilic and contained large acidophilic granules. The nuclei were large, found [?round], and clear but contained very little chromatin, so that they stained faintly. In a number of nuclei were found mitotic figures of such character that they could not be associated with protozoa.

"A second type of body present in the alveoli was similar to toxoplasma and was characterized by acidophilic cytoplasm containing numerous small acidophilic droplets and definitely basophilic nuclei of small size. This body was uniformly multicellular. A third type was a multicellular body containing homogeneous cytoplasm with small, definitely basophilic nuclei.

"Gram-Weigert stains were made of a number of sections showing the various types of unknown body. From study of the sections no evidence was derived to indicate that the bodies were similar in nature to histoplasm or that they were bacterial organisms. They could not be identified as protozoa. Authoritative opinion indicates that they were not worm eggs. The nature of these abnormal bodies is still under investigation."

Microscopic sections of about 100 hares were examined during the course of our study, and bodies satisfying the description given by Green and Shillinger have been observed frequently in the alveoli of infected animals. Comparison of these preparations with similar material from the lungs of deer with protostrongylin infections and sheep with *Muellerius capillaris* infection has convinced us that the bodies in question, contrary to the opinion of Green and Shillinger, represent lungworm eggs in various stages of segmentation. Their "small" worms doubtless were first stage larvae.

Although Boughton correctly noted that the eggs show no trace of segmentation at oviposition, some of his other statements seem to imply that a part of the development might take place in the feces. Our observations indicate that segmentation and hatching normally occur in the alveoli of the lung, as is characteristic of lungworms that deposit unembryonated eggs. The first stage larvae may be found in the alveoli, bronchioles, bronchi, trachea, and alimentary tract. We have never discovered lungworm eggs in the feces, but larvae are readily demonstrable in the pellets when they are present.

SUMMARY

1. The lungworm of the Varying hare in the eastern part of the United States and Canada constitutes a single species originally named *Synthetocaulus leporis* by Boughton (1932). It is here renamed *Protostrongylus boughtoni* nom. n., and a new diagnosis given for it.

2. This species is distinct from the species of *Protostrongylus* occurring in ruminants and also from the two species occurring in European hares, namely *P. kamenskyi* Shul'ts, 1930, and *P. pulmonalis* (Frölich, 1802) comb. n. (syn. *P. commutatus*).

3. The discussion by Green and Shillinger (1935b) on lungworm infection in hares of Minnesota and Wisconsin is quoted in full. Bodies observed but not iden-

tified by these authors are considered to be lungworm eggs in various stages of segmentation.

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THELASTOMA ICEMI (SCHWENCK), A NEMATODE OF COCKROACHES*

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In September, 1940, the writer obtained some specimens of the American cockroach, *Periplaneta americana* Linn., from Texas through the courtesy of Dr. Donald V. Moore of Rice Institute. Examination of these roaches revealed three species of nematodes. Two of the species were identified as *Hammerschmidtiella diesingi* (Hammerschmidt) and *Leidynema appendiculatum* (Leidy). The third species, previously reported only from South America, was identified as *Thelastoma icemi* (Schwenck, 1926) Travassos, 1929.

In January, 1942, after examination of approximately 250 specimens of the roach host from Nebraska, *T. icemi* was recovered in Lincoln; in October, 1942, the worm was recovered from the roach *Periplaneta brunnea* Brunn. in Louisiana. Both of these are new host records. The description of the writer's specimens of *T. icemi* is given below; the male of this nematode is here described for the first time.

Thelastoma icemi (Schwenck, 1926) Travassos, 1929

(Figs. 1–4)

With the characters of the genus as given by Chitwood (1932). (Measurements in mm)

Male: 1.1 long by 0.07 wide. Esophagus plus bulb 0.16 long; isthmus not distinct. Anterior portion of esophagus 0.13 long by 0.024 wide (at nerve ring); bulb 0.033 long by 0.034 wide. Nerve ring 0.10 from anterior end. Excretory pore 0.20 from anterior end. Intestine simple; anus 0.18 from posterior end of body. Tail filiform, bearing a pair of ventral preanal papillae, a pair of sublateral postanal papillae and a pair of ventral papillae 0.029 posterior to the anus. One spicule, 0.030 long.

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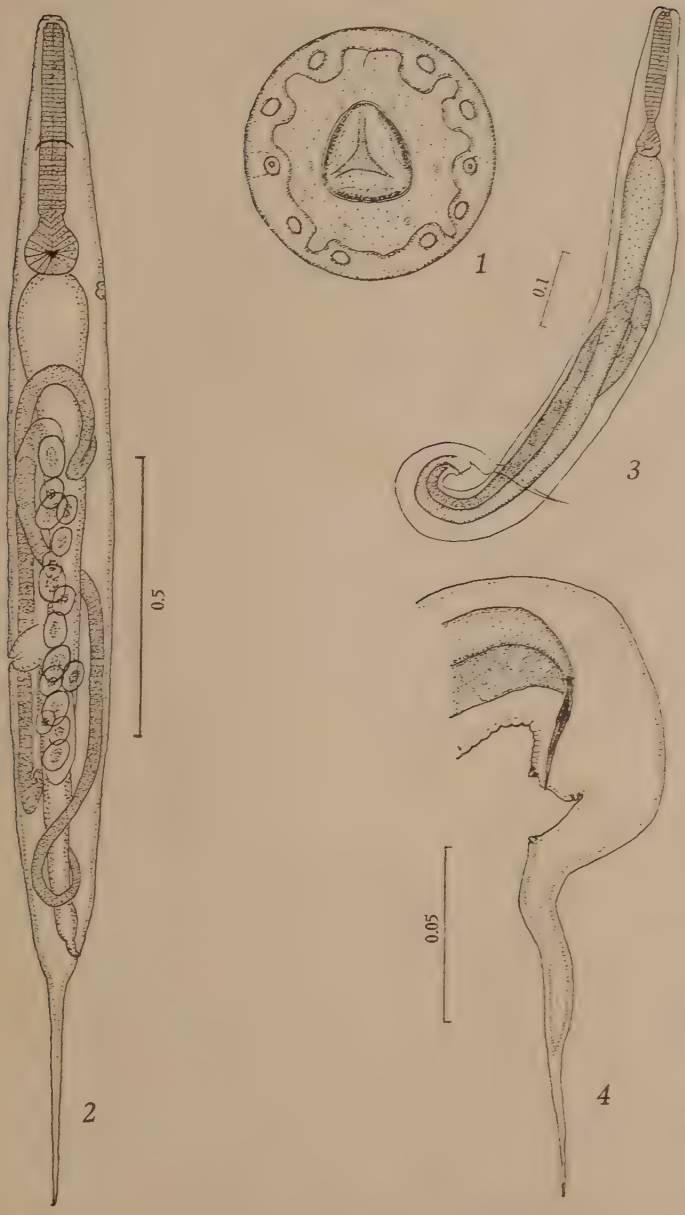
* Studies from the Zoological Laboratories, University of Nebraska, No. 218. The work was done under the direction of Dr. H. W. Manter.



Thelastoma icemi (Schwenck, 1926) Travassos, 1929

FIG. 1. Head of female, en face view.
FIG. 2. Adult female.
FIG. 3. Adult male.
FIG. 4. Tail of male.
Drawings made with the aid of a camera lucida.

Female: 2.3 to 2.8 long by 0.20 to 0.27 wide. Body distinctly annulated throughout. Tail slender, short, attenuated. Alae inconspicuous. Esophagus consisting of an anterior cylindrical portion separated from the bulb by a short but definite isthmus; esophagus plus bulb 0.37 to 0.42 long. Corpus 0.26 to 0.34 long by 0.035 to 0.037 wide (at nerve ring); isthmus 0.021 to 0.034 long by 0.033 to 0.042 wide; bulb 0.084 to 0.087 long by 0.087 to 0.096 wide, provided with a distinct valve. Intestine pronouncedly dilated at its anterior end. Nerve ring 0.17 to 0.21 from anterior end. Excretory pore 0.38 to 0.47 from anterior end or slightly posterior to base of esophagus. Vulva not salient, 1.22 to 1.30 from anterior end of body; reproductive system amphidelphic. Eggs ellipsoidal, 0.065 to 0.070 long by 0.050 to 0.057 wide, deposited before segmentation. Anus 0.5 to 0.6 from posterior end of body.

The measurements of the female specimens are, with the exception of the diameter of the esophageal bulb, in agreement with the extremes given by Schwenck (1926) in his diagnosis of *T. icemi*.

Chitwood (1932) felt the status of *T. icemi* as a member of the genus *Thelastoma* to be in doubt because the male had not been described, the excretory pore was posterior to the base of the esophagus, and the tail of the female was attenuate rather than filiform as in the type species. The male described in this paper closely resembles the males of *T. bulhoesi* and *T. riveroi* which are listed by Chitwood as valid species. *T. icemi* agrees with *T. magalhaesi* and *T. robustum* in having the excretory pore posterior to the base of the esophagus; such a character appears to the writer to have no more than specific value.

The genus *Thelastoma* was erected by Leidy (1853) when he raised it from subgeneric rank in the genus *Aorurus*. According to my investigations the genus at one time or other contained approximately 22 species whose hosts were reptiles and insects. Walton (1927) created the genus *Thelastomoides* to accommodate the species from reptiles. Recognition of synonymy and transference of species to other genera have reduced the number of species to 18.

Thelastoma icemi most closely resembles *T. magalhaesi* from which it has been adequately distinguished by Schwenck (1926) in his diagnoses of the two worms. *T. icemi* and *T. magalhaesi* differ from other members of the genus which occur in cockroaches in that the excretory pore is located slightly posterior to the base of the esophageal bulb.

SUMMARY

1. The occurrence of the nematode *Thelastoma icemi* in the cockroach *Periplaneta americana* (new host record) from Texas and Nebraska is here reported.

2. A second roach host, *Periplaneta brunnea* (new host record), is recorded from Louisiana.

3. The male of *Thelastoma icemi* is described for the first time.

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THE WANDERING OF *HAEMONCHUS* IN THE SHEEP HOST

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Some degree of wandering in the final host is characteristic of many parasitic nematode species. With *Ancylostoma caninum*, the dog hookworm, which is a skin penetrator, Otto and Kerr (1939), for instance, have used subcutaneously injected larvae as standard experimental procedure to establish intestinal infections. *Haemonchus contortus* does not infect by penetrating the skin, but Ransom and Foster (1920) reported: "The senior writer has observed the larvae of *Haemonchus contortus* (stomach worm of ruminants) in the lung of a guinea pig killed 48 hours after it had been fed a culture of the larvae, which indicates that they are able to migrate from the alimentary tract to the lungs and perhaps do so normally in their life cycle in their usual hosts, sheep, cattle, etc." Ransom seems not to have adduced any further observations on this possibility, although Chandler, Alicata and Chitwood (1941) evidently refer to Ransom and Foster (loc. cit.) when they mention concerning *Haemonchus contortus:* "Although there is no evidence that the worms perform a parenteral migration in sheep, Ransom (1920) showed that they *do* migrate to the lungs of guinea pigs."

Attempts made by the writer to confirm such an observation by administering larvae by mouth to rats, guinea pigs and rabbits have uniformly failed. Recently, in the course of other experiments on sheep, the demonstration that *Haemonchus* is not a wandering form seems adequately proved. The potential bearing of this fact on explanations regarding the immunization of sheep against this form renders the observations of interest beyond the range of life history knowledge.

MATERIALS AND METHODS

Sheep hosts.—All but five were captured at birth and bottle-fed on cow's milk (Klim) as described by Smith and Ring (1927). When used in the present experiments the five sucklings and four of the bottle-fed lambs had contaminating infections with *Strongyloides papillosus*. The others when used were helminth-free at ages of 7 to 70 weeks, as determined by fecal examinations and cultures (Stoll, 1930).

Infective Haemonchus *larvae.*—By making collections of feces in a paraffined paper box directly from the rectum of a sheep in which a pure infection of this nematode has been established, larvae could be readily cultured. The "seed" strain during the present experiments was in its 9th–14th passage, and demonstrably pure at every autopsy where *Haemonchus* was used as the solitary infection. Infective larvae were isolated in the Baermann apparatus from cultures 7 to 10 days old, thoroughly washed in several changes of tap water and then exsheathed and rendered bacteria-free as earlier described (Glaser and Stoll, 1940; Stoll, 1940). Such larvae are conveniently referred to as "axenic," Baker and Ferguson (1942) having recently applied this adjective to describe "a living organism that is free from all other demonstrable organisms."

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Injection of axenic larvae.—The larvae during sterilization were in dilute Tyrode solution or well water, the latter at this laboratory approximating a dilute "balanced" salt solution (Stoll, 1940). They were injected within a day after completion of sterilization, and in each case were confirmed as active and vigorous. For intraperitoneal injections, it was found useful for an assistant to hold the animal in an upended position with the head down, causing the viscera to slump toward the diaphragm. The larvae, contained in 4 to 7 ml of suspension, were then injected in the right posterior abdominal quadrant just off the midline. In the case of H38, receiving 1,300,000 larvae, 16 ml was injected. For subcutaneous injections 2 to 4 ml were used. Injections were performed with regard to maintaining sterility, using No. 18 needles. The skin area was sponged freely with alcohol, usually followed by iodine solution, and iodine was used again after injection, particularly to kill any larvae which might have adhered to the skin surface on withdrawal of the needle.

Local skin abscesses resulting from contamination during injection were notably absent, but did occur and persist in one animal, H97, of the intraperitoneal series, which struggled during the injection.

EXPERIMENTS

The procedure employed was to inject axenic infective *Haemonchus* larvae parenterally into the normal host and determine whether the nematodes under such conditions established themselves as parasites in their normal site, the 4th stomach or abomasum.

In preliminary experiments it was demonstrated that axenic exsheathed *Haemonchus* larvae were capable of infecting when administered by the normal route.

1. *Infectivity of exsheathed, axenic larvae given by mouth.*—Infective *Haemonchus* larvae as ingested under natural conditions are characteristically sheathed, that is, the 3rd stage larvae are still enclosed in the cuticular coat of the 2nd stage. Ecdysis normally occurs before the larvae reach the abomasum. Can larvae already exsheathed and rendered axenic before administration by mouth establish an infection?

To ram H9, 13 months old, 5200 axenic larvae were given by mouth. Eggs in the feces were 12 per gram on the 19th, 200 per gram on the 22nd day of infection. Five days later the animal was killed and 322 adult *Haemonchus* (of which 179 were female) recovered from the abomasum.

To wether lamb J59, 7 weeks old, 25,000 axenic larvae were given by mouth. At the first examination on the 13th day the feces gave a positive test for occult blood, which was increased in amount on succeeding examinations until the lamb, severely anemic, died on the 19th day, without eggs of the parasite yet having appeared in the feces. From the abomasum 7020 *Haemonchus* were recovered.

These two animals demonstrate that *Haemonchus* larvae rendered axenic have not thereby lost their ability to infect, and, when sufficient numbers are present in their normal habitat, to produce death.

The cases of 5 animals discussed in the next section (H33, H37, H96, H52, and H97) are also interpreted as evidence of the ability of axenic larvae to normally establish an infection when introduced directly into the rumen. H96 and H97 were especially of interest in this connection.

2. *Intraperitoneal injection of axenic larvae.*—The large size of the rumen in sheep crowds the other abdominal organs and makes the abdominal skin tight. Injection into the peritoneal cavity thus presents the hazard of penetrating the rumen itself. Some of our tests demonstrate this and constitute an experimental error rather than the desired test. However, as placement of the larvae within the abdominal cavity presents them with the most direct opportunity to penetrate the rumen or stomach wall if they can, even one failure to do so after injection into the peritoneal cavity, becomes a fact of considerable significance, from the standpoint of ability of *Haemonchus* to wander or migrate within the host.

H38, a wether 15 months old, was injected with 1,300,000 larvae. Three days later when the animal was killed, numerous "cysts" containing larvae were found in the mesentery and omentum, but the abomasum, though examined with great care, revealed not one *Haemonchus*.

H85, a wether 5 months old, was injected with 286,000 larvae. Six days later when the animal was killed, numerous small pinkish nodules, up to 5 mm in diameter, were found on the omentum and on the rumen wall. The abomasum was examined with great care and not one specimen of *Haemonchus* found.

Fresh preparations of these peritoneal nodules from the two animals showed numerous trapped larvae, but none of them exhibited signs of the first parasitic ecdysis or development into the 4th larval stage. Those from H38, killed 3 days after injection, were for the most part still alive, although some were disintegrating. The proportions were reversed at 6 days in H85. Individual larvae could be seen entirely encased in wandering cells against which they vainly struggled. In these two tests there was no sign whatever that intraperitoneally injected larvae could reach their normal site in the abomasum. Instead they were trapped and being killed off by the peritoneal reaction.

Three sheep were given single intraperitoneal injections and the fate of the infections followed by fecal examination. H81, a ram 4 months old, was injected with 406,000 larvae, and its twin ewe H82 similarly given 81,000 larvae. They remained negative during 11 weeks of fecal examinations. H95, a ram 8 months old, was injected with 454,000 larvae and remained negative during 7 weeks of fecal examinations. All three of these animals became positive when given larvae by the oral route.

The above animals each received a single injection of larvae. Divided doses were also tried. H89, a wether 8 months old, was given a total of 488,000 larvae in 4 doses of about 122,000 each, injected at weekly intervals. Four weeks after the last injection (being 7 weeks after the first), fecal examinations having continued negative, a test dose of larvae was administered by mouth. Through 4 additional weeks no eggs were demonstrable, and then the animal became patent as a result of the oral infection.

The 6 animals above reported represent adequate evidence of failure of intraperitoneally injected larvae to migrate and establish themselves in the abomasum. The records of 5 others are given in which larvae did reach the abomasum, evidently through accidental penetration of the rumen wall by the injecting needle. Two, H33, a ewe, and H37 a wether, each 13 months old, given respectively 209,000 and 355,000 larvae, were positive at fecal examinations on the 34th day, not having been examined earlier. These two animals, the first of the series, were injected while

prone. H96, a ewe 8 months old (and twin to H95 above), was given 108,000 larvae, but it was realized at the time of injection that a hollow organ, presumably the rumen, had been entered. Negative to fecal examination on the 15th day, she was positive on the 17th, and 6 days later reached a count of 35,400 eggs per gram, representing about 22 million per day. H52, a ram 16 months old, was injected with 434,000 larvae. Twelve days later the animal was killed. In an area of about 50 square centimeters in the omentum were numerous firm, pinkish nodules up to 3 mm in diameter. While *Haemonchus* larvae could, with difficulty, be distinguished in these microscopically, none of the worms showed movement or development. On the rumen wall was an area 1 cm in diameter which similarly showed reaction to the presence of *Haemonchus* larvae, as well as evidence of earlier trauma. On examination of the abomasum 60,300 *Haemonchus* were recovered, all in the 4th larval stage, in size up to 4.9 mm long. A 5th animal became positive after "intraperitoneal" injection, under circumstances indicating clearly that such result was direct inoculation into the rumen. H97, a ewe 8 months old, was injected with 112,000 larvae in four approximately equal doses of 28,000 each, spaced weekly. This animal struggled at the time of the first injection, a small abscess later developing at site of puncture of abdominal wall. Negative 15 days after the first injection, H97 showed *Haemonchus* eggs at 17 days. In number these remained very few for a period of 25 days and then rose slightly, reaching 600 eggs per gram 6 weeks after the initial injection (3 weeks after the 4th injection) and 2200 per gram a week later. In the case of H97, evidently there was some injection directly into the rumen at the 1st and 4th injections. However, failure of the egg count to increase until an interval appropriate for the 4th injection, indicated no increase in the abomasal infection until then, i.e., no penetration into the stomach by worms reaching only the peritoneal cavity on the 2nd and 3rd injections. None of these five exceptional cases furnish indubitable support to the successful wandering of intraperitoneally injected larvae to their home in the abomasum, as evidence in each instance suggests instead direct inoculation of larvae into some anterior portion of the gastro-intestinal tract.

3. *Subcutaneous injection of axenic larvae.*—Axenic larvae were next introduced subcutaneously, in order to avoid the occasional accidental inoculation into the anterior portion of the alimentary tract which occurred when injecting them intraperitoneally. In 24 of these tests the wool-free, inner aspect of one or both hind legs was used as the site of injection; in 2 additional lambs the larvae were given subcutaneously in the neck region.

TABLE 1.—*Results of single subcutaneous injections of 26 sheep and lambs with axenic* Haemonchus *larvae. One was negative for nematodes at autopsy 7 weeks after injection; each of the other 25 became positive after normal* Haemonchus *infection by mouth*

No. of animals	Age	Larvae injected	Examination periods after injection: weeks	No. of fecal examinations per animal	Results
3 sheep	12 months	119,000–147,000 (Av. 133,000)	7–9 (Av. 8)	12–17 (Av. 15)	Negative
5 lambs	20–23 weeks	55,000–80,000 (Av. 65,000)	3–11 (Av. 7)	4–7 (Av. 5)	"
4 "	14–19 "	49,000–61,000 (Av. 53,000)	6–11 (Av. 9)	4–7 (Av. 6)	"
12 "	6–9 "	24,000–47,000 (Av. 39,000)	5–9 (Av. 8)	7–16 (Av. 9)	"
2 "	2–3 "	28,000; 40,000	4; 8	3; 9	"

The results are summarized in Table 1. One of the lambs, J49, 7 weeks of age when injected with 21,000 larvae in 2 ml suspension in both right and left legs, was negative at weekly fecal examinations for 7 weeks. Then killed, no *Haemonchus* were found, the stomach being examined with special care. The remaining 25 animals were subsequently exposed to *Haemonchus* infections by mouth and became positive. Preceding the patency induced by the oral infections, they remained negative to fecal examinations for 3 to 11 weeks. During this non-patent period they were examined 220 times by floatation or culture, 3 to 17 times per animal.

In addition to these 26 tests, a month-old Guernsey bull was given 145,000 axenic larvae subcutaneously, aliquot portions being injected under the skin of each hind leg and the neck. The calf likewise remained negative to fecal examinations.

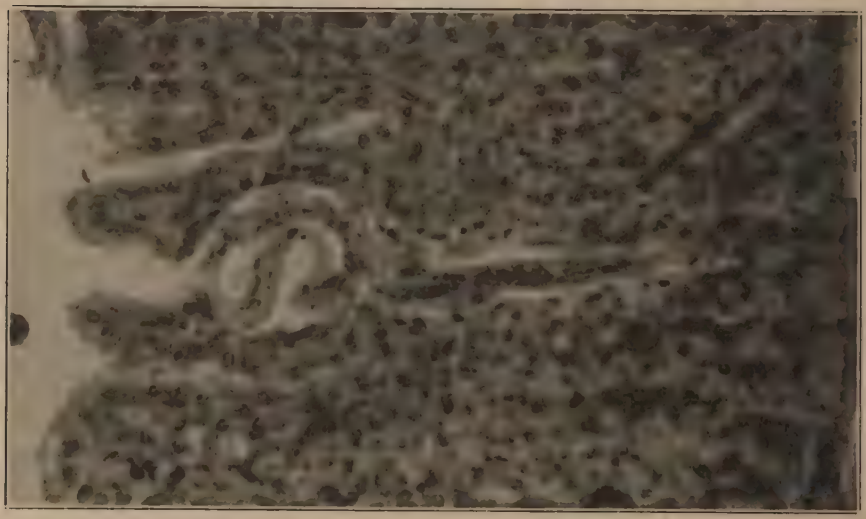
This consistent series of negative results following the injection of axenic larvae by the subcutaneous route into susceptible, normal hosts must be accounted a rigorous test of their inability to wander.

Pseudo-Migration by Haemonchus *in the Abomasum*

While *Haemonchus* is not to be classed as a wandering nematode in its final host, there is a minor degree of migration during the early establishment of larvae in the abomasum after normal infection by mouth. This pseudo-migration is undertaken by the 3rd stage larvae, which have usually lost their 2nd stage sheaths by the time they reach the 4th stomach. Here, in Veglia's (1916) words, "they lodge between the minute processes of the mucosa, where they shelter without actually piercing the mucosa."

Some favorable material obtained from animals reared helminth-free and killed within 2 days after being given a single heavy infection of larvae by mouth, has permitted a better understanding of this first phase of abomasal parasitism by *Haemonchus*. A portion of an abomasal "leaf" examined in physiological saline promptly after killing of the host, shows striking differences in animals infected 12, 27, and 40 hours previously. In a 12-hour infection the larvae are still predominantly on the surface of the mucosa. They may be seen there with the aid of the low power binocular microscope and they may be washed off rather easily. In a 27-hour infection, some larvae are visible on the surface, but most of them are beneath it. Within a few minutes in warm saline they can be seen escaping from a cut edge in great numbers. In such stomach tissue fixed promptly and sectioned, they are characteristically observed in the gastric pits, which receive the tubules of the glands. The larvae, usually coiled like a watch spring, are somewhat larger than the pits and their presence increases the size of the cavity each occupies. A few show the tail extruded to the musocal surface. In general, however, they are entirely hidden in the upper third or half of the mucosa, and may extend laterally a distance of 40 to 60 μ, more or less parallel to the surface.

While most of the larvae appear to remain coiled at the level of the gastric pits, occasionally one is seen to have penetrated the neck of a gland and gone deeper into its lumen. Of 74 unselected larvae traced from section to section individually, 12 or 16 per cent, had done this. In such case the anterior end of the larva may reach as far as the fundus of the gland, or even go on to nearly touch the muscularis mucosae. The larvae show no predilection for blood vessels at this stage. There is no extravasated blood near them, and little or no tissue reaction, which may be



FIGS. 1–2. Pseudo-migration of *Haemonchus contortus* larvae in abomasum of sheep 27 hours after normal infection by mouth. This is the 1st parasitic stage, passed in the gastric pits, but here the 1st parasitic ecdysis has not yet occurred. The three larvae in Fig. 1 (upper figure, with gastric lumen above; ×230) showed in neighboring sections no penetration deeper into the mucosa than here photographed. This characterizes the position of 84 per cent of those observed. The larva in Fig. 2 (lower figure, with gastric lumen at left; ×400) has the anterior end thrust deep into the tubule of a gastric gland.

Zenker fixation; haematoxylin-eosin stain; sections 9 microns. Photomicrographs by J. A. Carlile.

accounted additional evidence that they tend to accommodate themselves to the naturally available openings. Fig. 1 shows three larvae in section at 27 hours after infection, and Fig. 2 a single larva from the same host, the anterior end of which has penetrated deeply into a glandular tubule. In favorable sections at 27 hours the retraction of the 3rd stage sheath can be seen at the anterior tip of the larva, with the primitive capsule of the 4th stage larva already formed within, as in Fig. 3.

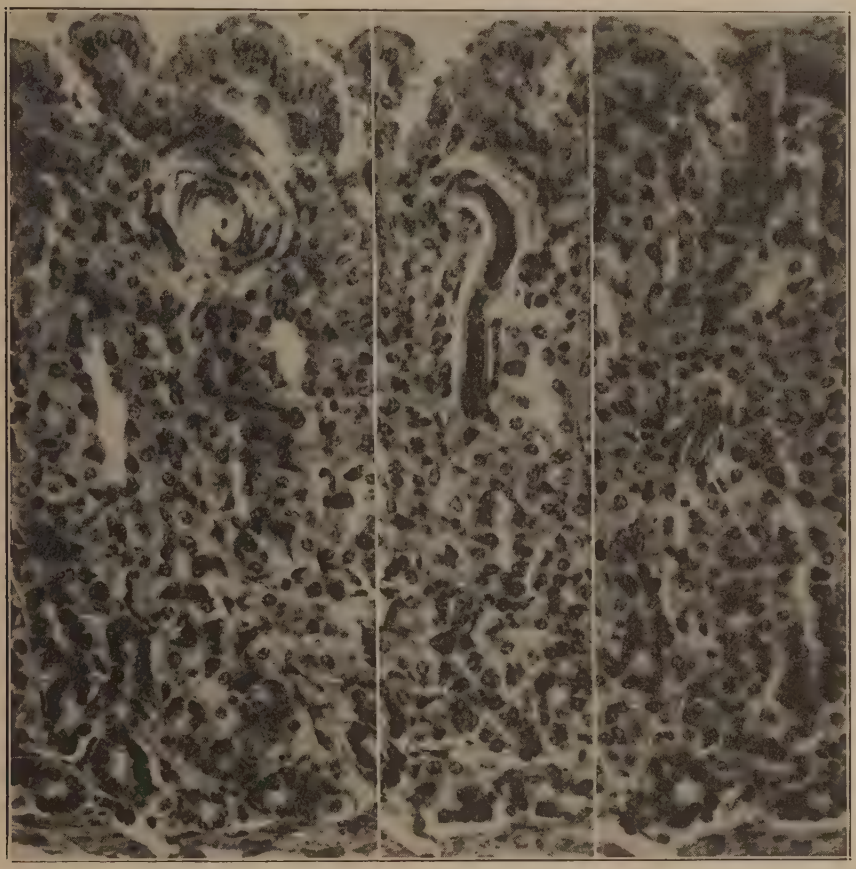


FIG. 3. Three successive sections (9 microns each) from abomasal leaf of sheep 27 hours after oral infection with *Haemonchus*. In this specimen the 1st parasitic (3rd larval) stage is near completion, separation of the sheath having begun at anterior end of larva (arrow).

Magnification ×400; Zenker fixation; haematoxylin-eosin stain. Photomicrographs by J. A. Carlile.

The photomicrographs are from a sheep which received an oral dose of 1,500,000 infective larvae. While the mucosa was extensively parasitized by this huge number of organisms, the number of gastric pits which had been entered by larvae represented only about one-thirtieth of the number of such openings. In a 40-hour infec-

tion occasional embedded larvae can still be observed, but the majority are again at the surface, and these are now characteristically young 4th stage larvae.

It will be recalled that the first parasitic ecdysis and emergence of 4th stage larvae can be obtained in vitro (Stoll, 1940) in the absence of nutrients. On this account it is doubtful whether Veglia's statement that "as soon as the mature larvae have shed their skins [i.e., 2nd stage sheaths] they begin to feed" can be regarded as established. The short migration into the gastric glands is more likely a requirement for the first parasitic ecdysis under conditions of lowered oxygen tension, as demonstrated in vitro. During this pseudo-migration there was, as noted, no evidence of blood vessels being traumatized, and stomach contents of a sheep—even after a 40-hour infection—gave no positive test for occult blood, although the infection was established with 535,000 larvae. The in vitro observation here parallels that of Veglia: "It appears consequently that but little or no growth occurs in the third stage. The object of this period of life seems to be the formation of a mouth apparatus adapted to piercing the mucosa of the stomach and thus to enable the larva to resume its parasitic life as a blood sucker." There is no difficulty in demonstrating blood in the abomasum when the 4th stage larvae or young adults are present in numbers.

DISCUSSION

As is well known, gastro-intestinal nematodes which wander in the final host enter blood vessels or lymph channels after penetrating the skin or the intestinal wall. Then carried passively to the right heart and to the lungs, they free themselves from the capillaries in the lungs and make their way via the alveoli, bronchioles, bronchi, and trachea to the back of the mouth where, caught in the swallowing movements of the host, they become re-routed down the food tube. Skin penetrators like hookworm and *Nippostrongylus muris* have been employed in standardized experiments to establish "normal" infections by using subcutaneous injections to introduce the larvae into the host (Otto and Kerr, 1939; Watt et al. 1943). That *Ascaris* can be shown to demonstrate the lung-wandering stage by introducing infective eggs into the mouth of a guinea pig is a common laboratory exercise.

The inability of *Haemonchus* as a skin penetrator has been recognized since Veglia's (1916) experiments. If *Haemonchus* possessed the ability to wander after parenteral injection, it must be assumed the species would demonstrate this when the opportunity was repeatedly presented. The fact that none of 26 sheep and lambs, and one calf, injected subcutaneously with a total of 1½ million larvae, showed any sign of infection established in the abomasum, would appear to be adequate evidence of inability to classify *Haemonchus* as a wandering form.

Of 6 animals in our series injected intraperitoneally with a total of over 3 million larvae, 2 examined at autopsy 3 and 6 days later, and 4 examined by fecal examinations for periods up to 11 weeks after the injections, showed no signs of *Haemonchus* reaching the abomasum. Two of these must be considered especially severe tests. In one case 1,300,000 were injected in a single dose. In the other, there were four successive weekly injections totalling nearly a half-million larvae. In neither of these was there found any sign whatever that larvae reached the abomasum. The former was negative at autopsy 3 days after injection, and the latter negative for a period of 11 weeks with 1 to 4 fecal examinations weekly.

Fertile *Haemonchus* infections were later established by the normal route in 31

of the 32 above ovine hosts. The 32nd was negative at autopsy when killed 7 weeks after injection. The influence of the preliminary parenteral larval injections is elsewhere noted (Stoll, 1942).

The fact that 5 other "intraperitoneally" injected sheep (one examined at autopsy 12 days later, 4 by fecal examinations only) did show establishment of worms is less readily explained as a demonstration of wandering of the larvae than of direct penetration of some one of the four stomachs of the sheep, presumably the rumen, by the inoculating needle. Ancillary data tend to confirm this presumption. In H52, which came to autopsy 12 days after injection, an area in the rumen wall, indicative of earlier trauma, was observed. H96 showed a high egg count at the usual interval appropriate for a mouth infection, and there was evidence at the time of injection that apparently the rumen had been entered. H97, injected four times at weekly intervals, showed egg counts appropriately explainable as experimental error in entering the rumen on the 1st and 4th injections, and no appropriate egg count rise for the 2nd and 3rd injections. Concerning the other two positives, H33 and H37, which were the first two animals injected, no further evaluation can be given due to their subsidiary use in another experiment, and their inadequate examination record.

For any one who has attempted intraperitoneal injections through the taut abdominal wall of sheep with materials whose fate, if reaching the lumen of a hollow organ, can be demonstrated there, the fact that penetration of the rumen should occasionally have occurred will not seem strange. McCoy (1935) has noted that injection of rats with live *Trichinella spiralis* larvae occasionally caused infections to become established, presumably because the needle accidentally penetrated the intestine of the rat.

It may be emphasized that if *Haemonchus* is a typical wanderer, even one animal injected parenterally and not showing subsequent infection would outweigh in significance several which did.

These findings are not in disagreement with the mild and transient degree of invasion of the glandular openings in the stomach wall by larvae under conditions of normal infection.

SUMMARY

Infective *Haemonchus* larvae, rendered axenic and injected subcutaneously into 26 sheep and 1 calf, failed to show establishment of infection in the abomasum. A similar failure to establish abomasal infections occurred after intraperitoneal injection of 6 sheep. The hosts ranged in age from 2 weeks to 13 months, the doses from 24,000 to 1,300,000. The series constitutes adequate evidence that this nematode is not a wandering form. Five additional "intraperitoneally" injected sheep later showed abomasal infection, interpreted as accidental injection of larvae directly into the rumen.

Lodgment of *Haemonchus* larvae in the gastric pits during the first parasitic stage following the normal oral route of infection is demonstrable but is not regarded as evidence of "wandering" in the usual sense.

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INCREASED INFECTIVITY OF THE EGGS OF THE DWARF TAPEWORM (*HYMENOLEPIS NANA* VAR. *FRATERNA*) FOLLOWING STORAGE IN HOST FECES[1]

JOHN E. LARSH, JR.[2]

In all previous quantitative work on *H. nana* var. *fraterna*, the eggs for infections were isolated from fecal pellets passed recently by infected animals. Shorb (1933) found that the percentage development of these eggs in rats and mice was exceedingly low, ranging from 0.0002 to 0.4 in rats, and from 0.1 to 1.0 in mice. However, Hunninen (1935) in his studies on mice reported much higher percentages of development, with that of cysticercoids ranging from 1.6 to 13.2, with an average of 4.1, and of adult worms from 1.0 to 8.0, with an average of 2.8 per cent. Some of Hearin's (1941) experiments with mice showed about a 3.4 per cent development of cysticercoids, and in a few preliminary experiments of the writer, approximately 4.3 per cent of the eggs administered to mice were observed later to have developed into cysticercoids. Since any practical method for increasing the percentage development of eggs would prove valuable in experimental work, it seemed worthwhile to consider this problem.

Shorb (l.c.) found that eggs isolated from the pellets and stored in tap water rapidly lost their infectivity for mice after three days and that none was infective after 10 days. It was on the basis of these results that he considered the eggs to be most infective immediately after passing from the host. Since water is an unnatural medium for the storage of the eggs, it seemed of interest to test the effect on their viability of storage in the fecal pellets.

Eight experiments were performed at various intervals. The first four of these involved the strain of tapeworm obtained from mice bought from a local dealer which will be designated as the M_1 strain. Another strain which will be called the M_2 strain was obtained from mice of a rural dealer (Larsh, 1943). The experimental procedure followed in the use of each strain was similar. Fecal pellets were collected from a large group of infected mice, and the material divided into two portions. One portion supplied eggs for infecting mice immediately, the other portion was stored for 48–72 hours at room temperature in 50-cc centrifuge tubes filled with tap water. Then eggs isolated from this material were used to infect a similar group of mice of the same age. Ninety-three hours after infection, the animals of both groups were sacrificed and a count made of the number of cysticercoids present. The results of the first four experiments with the M_1 strain are tabulated in Table 1; those of the other four experiments with the M_2 strain are shown in Table 2.

In the experiments with the M_1 strain, Table 1, the mice receiving eggs that had been stored three days in feces had about twice as many cysticercoids (8.8–12.8 per cent development) as those receiving eggs from fresh feces (4.5–6.1 per cent devel-

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opment). In the experiments with the M_2 strain, Table 2, the storage in feces likewise appeared to increase development although the percentage of development was not quite twice that with the fresh eggs, being respectively 4.4–6.4 per cent and 2.8–4.1 per cent. These two strains showed constant differences in their host infectivity. A discussion of their relations is reserved for another paper.

TABLE 1.—*Showing the percentage development of cysticercoids in mice two and one-half months old infected with unstored and stored eggs of the M_1 strain*

Experiment No.	No. of mice	Egg dose	Average No. of cysticercoids	Standard deviation of individual measurements	Percentage development
		A. Eggs isolated from fresh feces			
1	10	900	42.9	6.5	4.8
2	5	1500	67.0	5.0	4.5
3	10	2000	121.3	20.5	6.0
4	5	3000	182.8	10.8	6.1
		B. Eggs stored from 48 to 72 hours			
1	10	900	79.0	12.4	8.8
2	5	1500	163.2	11.7	10.9
3	10	2000	255.9	37.4	12.8
4	5	3000	374.8	19.9	12.5

SUMMARY

Eggs of *Hymenolepis nana* var. *fraterna* isolated from fecal pellets that had been stored in an aqueous medium at room temperature for 48–72 hours showed a greatly increased percentage development of cysticercoids in mice compared with eggs from fresh feces. No explanation is offered to account for this increase in infectivity, but it may be an indication of a natural incubation period heretofore not considered.

TABLE 2.—*Showing the percentage development of cysticercoids in mice two and one-half months old infected with unstored and stored eggs of the M_2 strain*

Experiment No.	No. of mice	Egg dose	Average No. of cysticercoids	Standard deviation of individual measurements	Percentage development
		A. Eggs isolated from fresh feces			
5	10	1200	34.4	13.1	2.8
6	5	1500	53.2	8.8	3.5
7	10	2000	78.5	7.4	3.9
8	5	3000	121.8	5.2	4.1
		B. Eggs stored from 48 to 72 hours			
5	10	1200	52.6	14.0	4.4
6	5	1500	78.8	10.5	5.3
7	10	2000	127.7	9.0	6.4
8	5	3000	180.0	7.4	6.0

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A NEW ECTOPARASITE (ACARINA: CHEYLETIDAE) FROM DOMESTIC RABBITS

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AND
G. F. AUGUSTSON
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During the past six years the Los Angeles Wildlife Disease Research Station, U. S. Fish and Wildlife Service, has conducted a careful search for ectoparasites from more than 3,000 domestic rabbits, *Oryctolagus cuniculus,* that have been brought in for observation. All of these came from rabbitries in California, more than half of them from southern California. Because of hutch construction and approved practices of rabbit husbandry, very few ectoparasites are ever found infesting rabbits raised in commercial rabbitries of southern California.

On December 4, 1942, a three-year-old, New Zealand White, male rabbit was presented to the Station by a nearby rabbit breeder for diagnosis and treatment. Approximately three months before this animal had been placed in a recently acquired, secondhand rabbit hutch. This hutch was not sterilized or disinfected before being used. The seller of the hutch claimed it had been idle for two months.

The first indication of any disease was a fine, powdery, meal-like scruff or dandruff which the owner noted in the fur. This spot was located on the dorsal midline approximately five centimeters behind the shoulder blades. The infested spot was roughly circular in shape and approximately five centimeters in diameter. Much of the under fur in this spot had loosened and fallen out. The guard hairs were less in number than in adjoining areas. A very fine, gray-white, slightly oily scale covered this area. This scale could easily convey to the clinician the impression of the early stages of favus. The skin beneath the scale deposits was red, tender to the touch and slightly eroded. No crusts were formed, neither were there any scratch wounds to indicate the rabbit had attempted to alleviate a local irritation. No other affected areas were found although the body and extremities were carefully examined. Other than the infested area the rabbit appeared normal in every respect. Clinical examinations of the scale, skin scrapings and fur from the affected area failed to demonstrate the presence of any fungi. These same materials did, however, reveal the presence of a mite. It was quite evident that this mite was carrying on its complete life cycle on the body of its host because adults, nymphs, larvae and eggs were found.

From this evidence and the following analysis of these mites they undoubtedly represent a group, heretofore unknown to science, of true mammalian ectoparasites. It is concluded here a new genus, of the family CHEYLETIDAE, is necessary to hold this interesting parasite.

Family Cheyletidae

Ewingella n. g.

Very small mites, body slightly longer than broad, lightly compressed dorso-ventrally; dorsal plate in anterior half only; legs short, all uniform in length and structure; coxae narrow, paired

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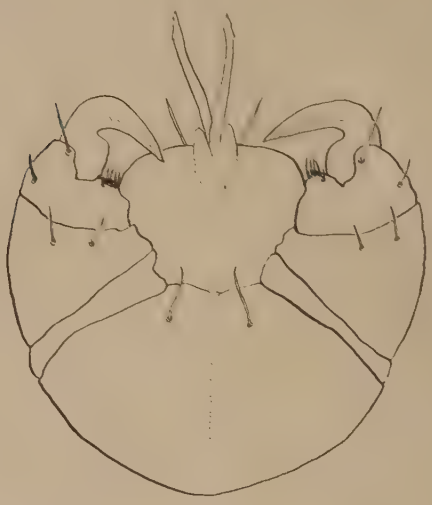

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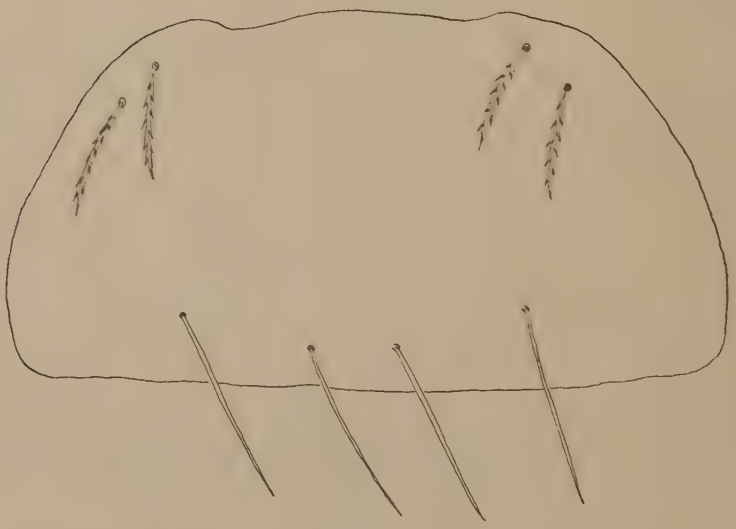

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FIG. 1. *Ewingella americana* n. sp., leg III, holotype female.
FIG. 2. *Ewingella americana* n. sp., capitulum, ventral, holotype female
FIG. 3. *Ewingella americana* n. sp., dorsal plate, holotype female

on a side I with II, III with IV, not joining any of those on the opposite side: chelicerae stylate, retractible; palpi very large, thick, arm-like, the last segment armed with a prominent, falcate claw, with minute opposing thumb; integument of entire body finely striated; setae simple to plumose; tarsi with single claw, all with a double pectinate appendage, analgeniyal aperture terminal; a dorsal, pseudo-division between cephalothorax and abdomen.

Genotype: Ewingella americana n. sp.

This new genus is nearest to *Cheyletiella*. It differs in the much broader palpi, type of tarsal claw, and, among other things, the single dorsal plate. The genus bears the name of Dr. H. E. Ewing, our foremost acarologist.

Ewingella americana n. sp.

(Figs. 1–3)

Holotype female:

Capitulum: palpi very thick at bases, armlike, opposing each other, terminal claws formidable, falcate, thumb minute, with three to four subequal setae, segment II with long, dorsal, plumose setae, segment III with shorter setae; hypostome and epistome fused, broadly cone-shaped, with fluted lateral trocheal openings; chelicerae stylate; basi capitulum broad, with two upper, sub-median, short setae.

Body Dorsum: slightly broader in anterior half; dorsal plate rectangular, widest posteriorly, with two plumose setae in each antero-lateral angle, four simple setae on posterior margin, surface finely pitted; lateral to dorsal plate two long plumose setae, also one on each shoulder just off dorsal plate; a fold in integument between cephalothorax and abdomen; posterior half of dorsum with two sub-median simple setae just below dorsal plate, two long plumose setae upper and lower on lateral margins, with few small setae in between, also around anal aperture; body terminates in two long bristles; venter with numerous small setae, of which only two in lower lateral angles are plumose; anal-genital aperture continued dorsally, vulva anterior to anus; coxae in pairs, somewhat radially arranged; legs uniform, with scattered plumose setae, as well as plain setae, taper slightly to tarsi, claws double pectinate as a broken feather on a short, non-segmented pedicel.

Holotype: one female on *Oryctolagus cuniculus*, Los Angeles Wildlife Disease Research Station, U. S. Fish and Wildlife Service, Los Angeles, California. Deposited in the Allan Hancock Foundation, University of Southern California, Los Angeles, California.

Paratypes: 12 females, collected and deposited as above.

Members of the mite family CHEYLETIDAE are for the most part predatory in nature, preying upon other mites and small insects, either attacking them directly or indirectly by feeding on their eggs. There are a few groups within this family which apparently lead a parasitic existence. One such group recently received a fine analysis by Ewing (1938). Unfortunately *Cheyletiella canadensis* Banks, 1909, a supposedly parasitic form, is little known, and from all indications the only representative from North America. As Ewing (ibid) has pointed out, much work remains in the study of cheyletid mites, particularly from North America. European workers, i.e., Oudemans, have been far in advance in regards to work completed on these important mites. Banks (1915, p. 28) records an interesting European cheyletid record. "*Cheyletus parasitivorax* Michiel uses the rabbit's fur as a hunting forest, where it destroys the *Listrophorus* mites which occur on the hairs of the rabbits." This is typical for the majority of cheyletids, other predatory forms are to be found almost everywhere, even on old agar culture plates, in nests of many birds, rats and mice, both native and domestic.

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RESEARCH NOTES

OBSERVATIONS ON A TRICHOMONAD FROM THE INTESTINE OF MAN

In the intestine of man trichomonad flagellates which possess five anterior flagella frequently occur (Wenrich, 1931; Arch. Soc. Biol. Montevideo, Suppl.: 1185–1204), and flagellates with four anterior flagella but otherwise identical have been reported to exist. The discrepancy in accounts of the number of anterior flagella may be the result of inaccurate observation or alteration of normal structure in the specimens examined, or may be due to genetic differentiation or factors in development giving real differences. The opinion is growing that the common trichomonad flagellates of the intestine of man belong to one species, which may be named *Pentatrichomonas hominis* (Davaine), as suggested by Wenrich (1931), or *Trichomonas hominis* Davaine, as used by Wenrich (1942, J. Parasitol. **28** Suppl.: 12). If this opinion is accepted, either the number of anterior flagella must be considered a variable characteristic, or reports of numbers other than five must be rejected. It is evidently on the basis of the former consideration that Wenrich (1942) stated that in *Trichomonas hominis* there are four or five anterior flagella. It is probable, in my opinion, that the complement of anterior flagella is a definite and stable characteristic in these trichomonads, as in other flagellates; if a four-flagellate form exists it deserves specific differentiation. This account will deal with a five-flagellate form found in a stool specimen sent into the laboratory by a person in California, and maintained in culture for eight months.

It is important that emphasis be given to the fact that the five anterior flagella are not in one group. It has been indicated by Kofoid and Swezy (1923, Univ. Calif. Publ. Zool. **20**: 373–390)

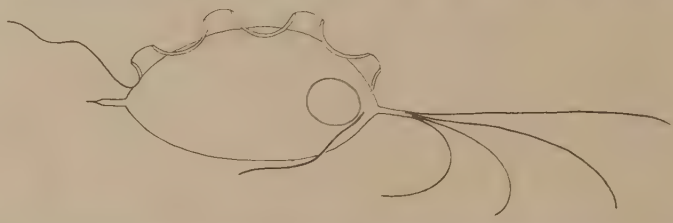

FIG. 1. *Pentatrichomonas hominis* (Davaine). Diagram of outline structure as demonstrated by darkfield illumination. ×3000.

and by Wenrich (1931) that there is a group of four flagella and an independent flagellum. By the use of darkfield illumination I have determined this number and arrangement of flagella in more than fifty living flagellates, which had been mounted under paraffin-sealed coverslips and left to become sufficiently slow in their movements. (Many were still very active after 24 hours at room temperature, and a few were alive after five days.) The group of four flagella originates at the anterior end of the body, its base appearing in darkfield as a columnar extension that shows only the two edges. After a length of 1 to 2 μ this column divides into four flagella. The length of the flagella in a group varies. Whatever the length of the body, which ranged in this material from 8 to 15 μ, the longest flagella measure 15 to 18 μ; the shortest are sometimes 8 or 10 μ. At one time in their course of movement they are turned back together against the ventral side of the body, and are at rest for an instant. The group is then thrown quickly across from the ventral to the dorsal side and rapidly forward; immediately, one following another, the flagella swing posteriorly to a position against the ventral side of the body. In this movement each flagellum becomes curved; when extended forward its concavity is toward the ventral side. The above description is of the flagellar movement seen in many specimens confined in one position and much less active than normal, but it probably indicates the pattern followed in the very rapid unmodified activity of the flagellate.

The independent flagellum appears by darkfield illumination to originate in a clear bright end about a micron posterior to the beginning of the whip of anterior flagella, on the side of the body opposite to the beginning of the undulating membrane. It is entirely separate from the flagella of the anterior whip, and from its origin is directed posteriorly, in the manner of a trailing flagellum. Its length varies from 6 to 13 μ; generally it is about half as long as the longest flagella of the anterior whip. It is moved backward and forward, usually in a limited arc, maintaining in general its posteriad direction on the ventral side. At times it is turned forward

or across the body, but it does not for the most part change position more than the free flagellum at the posterior end of the undulating membrane. The activity of the independent flagellum is much more like that of the membrane flagellum than like that of the flagellar whip, as was pointed out by Kofoid and Swezy (1923).

Some writers at the present time place all trichomonads with an undulating membrane into the genus *Trichomonas*, whether the anterior flagella number three, four, or four plus one (Wenrich, 1942). Differences in flagellar organization should be considered indicative of differentiation of groups of trichomonads, as well as of other flagellates. The trichomonad with which this report is concerned possesses a structure, the independent flagellum, which is a new addition, of a unique sort, to the organization that exists in *Trichomonas*. It is my opinion that it warrants generic distinction for the flagellate, and that the name *Pentatrichomonas* should be retained. It will never be known what was the structure of Davaine's flagellate, any more than of O. F. Müller's *Cercaria tenax*, except insofar as we can make plausible identification of their forms with flagellates that can be studied by modern methods. Nevertheless, there is probably as much justification for using the name *Pentatrichomonas hominis* (Davaine) for the common trichomonad of the intestine of man as for adopting *Trichomonas tenax* (O. F. M.) for the flagellate of the mouth of man.—Harold Kirby, *Department of Zoology, University of California.*

GIARDIA IN THE BLOOD OF A KANGAROO RAT

Various species of *Giardia* have been reported from a great variety of animals, usually inhabiting the duodenum or small intestine. There are very few reports of its occurrence elsewhere in the body of the host. Wenyon (1926, Protozoology) cites a report by Gonder, in 1910, of *Giardia* in blood films from a falcon shot in the Transvaal. Wenyon is of the opinion that this blood film was contaminated with intestinal material. He also cites Fantham's report, in 1919, of a *Giardia* from the blood, as well as the intestine of the South African silver fish. There have been several reports of the related genus *Hexamita* occurring in the blood. Recently Andrews (1941, J. Parasitol. **27** Suppl: 26) has reported it from experimental mice.

In February, 1942, a kangaroo rat (*Dipodomys heermanni*) was trapped on the San Joaquin Experimental Range, Madera County, California, and shipped to the laboratory of the U. S. Fish and Wildlife Service in Los Angeles. The animal was killed with ether. A blood smear was made from the heart and stained with Giemsa's stain. This thin film was a clean smear and showed no evidence of any possible contamination from the intestine. A single trophozoite of *Giardia* was observed in this blood film. It was similar in morphology to *Giardia* observed from the intestine of kangaroo rats.—Carlton M. Herman, *California Division of Fish and Game.*

COMPARING THE PERCENTAGE DEVELOPMENT OF THE DWARF TAPEWORM, *HYMENOLEPIS NANA* VAR. *FRATERNA*, OBTAINED FROM MICE OF TWO DIFFERENT LOCALITIES

In connection with a recent series of studies on the dwarf tapeworm, *H. nana* var. *fraterna*, in mice, data were obtained which suggest a physiological dissimilarity between two parasites isolated from mice of different localities. The first tapeworms were taken from white mice bought locally. In various experiments, a total of 50 mice, 2½ months old, were given initial infections and the resulting percentage development of cysticercoids ranged from 8.8, when 900 eggs were administered, to 12.8 following a test dose of 2000 eggs.

The first stock infection was lost during the summer of 1941 and another was established with worms from mice sold by a rural dealer. In all of the experiments performed to date, the percentages of cysticercoid development have been strikingly lower than those from similar infections with the first tapeworms. More than 150 mice, 2½ months old, have been tested as controls in various experiments in which infective egg doses varied from 900 to 3500. The range of cysticercoid development was from 4.2 to 6.6 which is about one-half that in mice of this age infected with eggs from the previous stock. Other infections in mice of other ages showed constant differences between these two parasites.

It is important to add that the percentages given for either of these parasites can not be compared directly with those of previous workers, because a new method of fecal storage was used to obtain eggs for infections (Larsh, 1943, J. Parasitol. **29**: 417–418). By this procedure, it was demonstrated that the percentage development of eggs into cysticercoids is consistently higher than that obtained with eggs from fresh feces. It is interesting to note that eggs of the second stock used for infections before storage, i.e., isolated from fresh feces, showed about the same percentage developments as those of a parasite obtained earlier from the same source by Hunninen (1935, Am. J. Hyg. **22**: 414–443). The average percentage in his study was about

4.1, whereas that of the unstored eggs in the present study was approximately 3.8. In all probability, the parasite used in both cases was of the same line. Heretofore, the differences in percentage development reported by various workers using the mouse strain of *H. nana* in mice have been attributed to personal factors, but perhaps they were due, after all, to slightly different strains of the parasite.

The results given above suggest that the two parasites studied are different physiologically. Just how much they differ can not be ascertained except by direct comparisons. Shorb (1935, Am. J. Hyg. **18**: 74–113) demonstrated two distinct physiological strains of this parasite, one in rats, the other in mice. The finding in addition of marked variations in the mouse form would seem to suggest that different strains of this parasite are produced easily and may be quite numerous. In a recent paper (Rappaport, 1943, Am. J. Trop. Med. **23**: 343–367) similar findings are reported for *Trichinella spiralis*.—JOHN E. LARSH, JR., *School of Hygiene and Public Health, Johns Hopkins University.*

A NOTE ON THE LIFE CYCLE OF *TAMERLANEA BRAGAI* SANTOS, 1934 (TREMATODA: EUCOTYLIDAE)

Subulina octona, a common land snail in Puerto Rico, has proved to be a satisfactory intermediate host of *Tamerlanea bragai* Santos, 1934, a kidney fluke of the domestic pigeon, *Columba livia domestica.* This trematode was described by Santos from Brazil (Reis and Nobrega, 1936, Doenças das Aves, Sao Paulo, Fig. 222, p. 313) and reported from Puerto Rico by Maldonado and Hoffman (1941, J. Parasitol. **27**: 91).

Preliminary observations indicate that the ova of the parasite are ingested by the snail. Mother sporocysts give rise to delicate, spherical daughter sporocysts. The daughter sporocysts give rise to an average of eight to ten tailless cercariae which at due time encyst inside the sporocyst. The cycle in the intermediate host is completed within two months after ingestion of the ova. Pigeons become infected by feeding on the infected snail. The parasite reaches the adult stage in about two weeks after ingestion of the encysted cercaria.

All the cercariae observed possess a well developed acetabulum. This organ, however, atrophies during development in the final host, but occasionally may be retained in a rudimentary form by the adult. It is suggested that because of the presence of an acetabulum in the cercaria and adults, *Tamerlanea bragai* should be excluded from the group of monostomes in which it is classified at present and included under the suborder Distomata.

A detailed study of the development of the parasite in the snail and final host is under way.—JOSÉ F. MALDONADO, *Department of Medical Zoology, School of Tropical Medicine, San Juan, P. R.*

CYSTICERCUS OF *TAENIA TAENIAEFORMIS* WITH TWO STROBILAE

One of seven strobilocercus larvae of *Taenia taeniaeformis* removed from the liver of a muskrat (*Ondatra zibethica*) captured on the University of Michigan campus, April 14, 1942, was abnormal in that two fully developed strobilocerci, 24 mm in length, were attached to a single

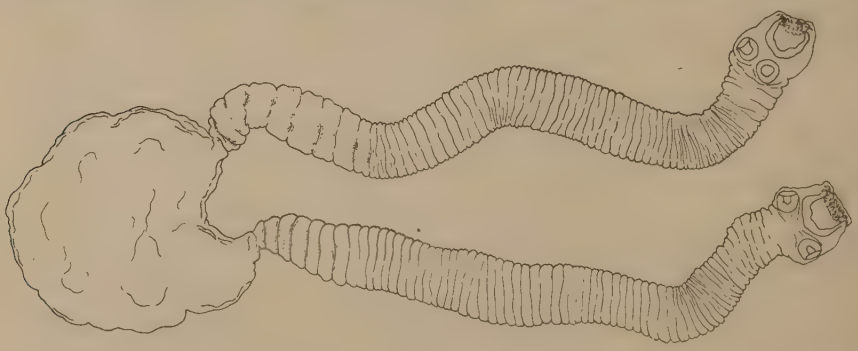

FIG. 1.

bladder, 5×7 mm. (See Fig. 1.) They were entwined around one another and tied into a rather compact knot. These two individuals were of equal size and even larger than some of the other cysticerci removed from the same liver. Each showed normal segmentation, suckers and hooks.

Although anomalies have been reported for larvae of this genus, none seem to have been recorded from North America. Southwell and Kirshner (1937, Ann. Trop. Med. and Parasitol. **31**: 37–42) published a description of a polycephalic cestode larva taken from the surface of the spleen of *Mastomys erythroleucus* (a rat) from Sierra Leone, West Africa, which they believed to be *Cysticercus fasciolaris*. In this specimen 12 segmented strobila measuring 20–22 mm in length arose from a single bladder (10 mm in diameter). Dollfus (1938, Ann. Parasitol. **16**: 133–141) has described a larva identified as *Taenia taeniaeformis* possessing a single bladder (7×13 mm) with 6 strobila, 10–12 mm in length, attached. The latter polycephalic anomaly was dissected from a white mouse from the French Cameroons, West Africa. More recently Lent (1942, Rev. Brasil Biol. **2**: 197–201) has reported the finding of four cysticerci of *Taenia solium* with two scolices each.

The development of more than one individual from a bladder in a species where normally one occurs, indicates multiple centers of growth in the bladder. Thus it would seem that the developing bladder worm may at times possess germinal properties characteristic of *Multiceps* or *Echinococcus*. This seems to accord with Dew's explanation of the origin and development of daughter cysts in *Echinococcus granulosus*. Dew (1925, Med. J. Australia **2**: 496–505) considers that the development of daughter cysts is the result of injury of an original hydatid, this injury being chemical, physical or by growth of structures of the surrounding host tissue into the hydatid cyst. These twinned cysticerci of *Taenia* as well as the polycephalic ones may have arisen as a result of injury or disturbance in the early development of their germinal layers. In the present example twinning has gone to completion whereas in tri-radiate, tetra-radiate or penta-radiate worms (Foster, 1915, J. Parasitol. **2**: 7–20; Cram, 1926, J. Parasitol. **14**: 54–55; Dobrovolny & Dobrovolny, 1935, Tr. Am. Micr. Soc. **54**: 22–27) a similar condition exists but complete separation of individuals has not been accomplished. It is surprising that more cases have not been reported.—Robert E. Kuntz, *Department of Zoology, University of Michigan, Ann Arbor, Michigan.*

ADDITIONAL RECORDS OF HUMAN INTESTINAL MYIASIS CAUSED BY *ERISTALIS*

Swartzwelder and Cali, 1942 (Am. J. Trop. Med. **22**: 159–163), summarized the reports of human myiasis caused by larvae of syrphid flies. Of 22 cases in the literature, including one reported by these authors, two were ascribed to the genus *Syrphus* and one to *Helophilus pendulus*. All the others presumably belonged to the genus *Eristalis*, or possibly *Helophilus*.

Three hitherto unreported cases of human infection with rat-tailed maggots of the genus *Eristalis* have come to my attention, as follows: (1) a full-grown *Eristalis* larva, the body portion 25 mm long, passed by a child 4 years of age at Tomball, Texas, on July 15, 1941; (2) an *Eristalis* pupa with body 14 mm long passed by a boy 6 years of age at Houston, Texas, on August 17, 1939; and (3) an *Eristalis* larva 18 mm long passed by a child 5 years of age at Houston, Texas, on August 11, 1943.

In each case a single specimen was passed in the feces alive, but failed to continue development when placed in water with decaying vegetation. No additional specimens were passed when anthelmintics or purges were given. In the first case mentioned above vague symptoms of abdominal discomfort were reported, but in the other two cases there were no symptoms referable to the infection.—Asa C. Chandler, *Rice Institute, Houston, Texas.*

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1933	William H. Taliaferro	Fred C. Bishopp
1934	Ernest E. Tyzzer	James E. Ackert
1935	Charles F. Craig	Harley J. Van Cleave
1936	Robert Hegner*	William B. Herms
1937	George R. LaRue	David H. Wenrich
1938	Fred C. Bishopp	Elery R. Becker
1939	Horace W. Stunkard	Henry E. Meleney
1940	David H. Wenrich.	Gotthold Steiner
1941	James E. Ackert	Justin Andrews
1942	Henry E. Meleney	Rudolf W. Glaser

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Norman R. Stoll	1930; 1931; 1932

Secretary

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Oliver R. McCoy	1938–39; 1940–41; 1942
James T. Culbertson	1942–

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Gilbert F. Otto	1937–38; 1939–40
L. E. Rozeboom	1941–42

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Charles A. Kofoid	1925	D. H. Wenrich	1932–35; 1936
B. H. Ransom*	1925	H. E. Meleney	1933–36
William A. Riley	1925–26; 1928–30	Norman R. Stoll	1933–36; 1937–40; 1941
Charles W. Stiles*	1925; 1929–32	Eloise B. Cram	1934–37
Ernest E. Tyzzer	1925–26	Wilbur A. Sawyer	1934–37
Maurice C. Hall*	1926–29	James E. Ackert	1935–38
Wilson G. Smillie	1926–27	Earl C. O'Roke	1936–39
Henry B. Ward	1926–29	Justin Andrews	1937–40
Franklin D. Barker*	1927–30	Harley J. Van Cleave	1938–41
J. H. St. John*	1927–28	Elery R. Becker	1939–42
W. H. Taliaferro	1928–31	Emmet W. Price	1939–42
Asa C. Chandler	1929–30; 1936–39	Clay G. Huff	1940–
W. B. Herms	1930–33	Horace W. Stunkard	1940–
Benjamin Schwartz	1930–33	Donald L. Augustine	1941–
L. R. Cleveland	1931	Raymond M. Cable	1942–
W. W. Cort	1931–34; 1935–38	Gilbert F. Otto	1942–
	Willard H. Wright 1942–		

* Deceased.

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ROBERT HEGNER*	1932–34	WILLIAM A. RILEY	1934–37; 1938–42
FRANCIS M. ROOT*	1932–34	WILLIAM H. TALIAFERRO	1934–37; 1938–42

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HENRY B. WARD	1932–33	OLIVER R. MCCOY	1936–39
ASA C. CHANDLER	1932–34; 1935–38; 1939–42	HENRY E. EWING	1937–40
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BENJAMIN SCHWARTZ	1933–37; 1938–41; 1942–	JUSTUS F. MUELLER	1941–
		HAROLD KIRBY	1942–

* Deceased.

‡ Beginning in 1942, the Chairman, Editorial Committee, became ex officio member of Council.

List of Meeting Places

1925	Kansas City	1931	New Orleans	1938	Richmond
1926	Philadelphia	1932	Atlantic City	1939	Columbus
1927	Nashville	1933	Boston	1940	Philadelphia
1928	New York	1934	Pittsburgh	1941	Dallas
1929	Des Moines	1935	St. Louis	1942	(New York, cancelled)
1930	Cleveland	1936	Atlantic City	1943	(No meeting)
		1937	Indianapolis		

AMERICAN SOCIETY OF PARASITOLOGISTS

CONSTITUTION

(Including revisions of Dec. 30, 1931, Dec. 28, 1932, Dec. 29, 1933, Dec. 31, 1940 and Dec. 30, 1941.)

NAME AND OBJECT

The name of the society is the American Society of Parasitologists.

The object of the society is the association of workers in the field of Parasitology for the presentation and discussion of new or important facts and problems in that science and for the adoption of such measures as will tend to the advancement of parasitological teaching and investigation in this country.

MEMBERSHIP

The members of the society shall be of two classes, active and foreign honorary.

Any person interested in parasitology may be a candidate for active membership.

Any foreign scientist who has made eminent contribution to Parasitology may be eligible for honorary membership.

Candidates for membership shall be elected by the Council.

OFFICERS

The officers of the society shall be a President and a Vice-President, who shall be elected for one year; a Secretary and a Treasurer, who shall be elected for two years; and members at large of the Council.

The Council shall consist of the President, the Vice-President, the Secretary, the Treasurer, the Chairman of the Editorial Committee, and eight members elected by ballot from the society at large, two for four years, two for three years, two for two years and two for one year. After the first year two members at large of the Council shall be elected each year to serve four years.

If any vacancy occurs among the officers, the Council is authorized to appoint a member to fill out the unexpired portion of the current year.

The routine business of the society shall be administered by the Council.

Five shall constitute a quorum of the Council.

DUES

The dues, to include subscription to the JOURNAL OF PARASITOLOGY, shall be four dollars the year for active members, unless changed by vote of the society.

MEETINGS

There shall be an annual meeting and such other scientific or business meetings as the Council shall determine.

During the annual meeting a business meeting will be held for the election of officers for the ensuing year and for the transaction of other business.

JOURNAL OF PARASITOLOGY

This Journal, the property of the society, is its official organ. Responsibility for its conduct shall rest with the Council which shall select the editorial staff and set the price of subscription.

ENDOWMENT FUND

Provision is made for the establishment of a permanent endowment fund, the principal of which may be expended only by a three-fourths vote of all members of the Council and approval by a three-fourths vote of the members of the society present at a regular meeting. The Council shall be entrusted with the maintenance of the fund, and the use of the income therefrom.

AMENDMENT

On recommendation of a two-thirds vote of the Council, the constitution may be amended by a two-thirds vote of the members present at any regular business meeting of the society, provided that at least 30 days' notice has been given to the membership of the proposed amendment.

BY-LAWS

MEMBERSHIP

1. *Election of Members.* An affirmative vote of all Council members present at a meeting shall be necessary for the election of candidates for membership. If the vote is taken by mail ballot, an affirmative vote of all members of the Council replying within thirty days shall be required.

2. *Members in Good Standing.* Members in good standing are those whose current dues are paid.

3. *Delinquent Members.* The JOURNAL OF PARASITOLOGY shall not be sent to members in arrears, and members in arrears for the current year and two previous years shall be dropped from the roll of the society at the end of the current year.

4. *Reinstatement of Delinquent Members.* Members dropped for non-payment of dues, or who have resigned, may be reinstated by the payment of all dues in arrears. Otherwise, the applicant must apply for election as a new member.

5. *Non-subscribing Members.* Where a second membership in the society is taken in the same immediate family, the second member may join upon the payment of annual dues of one dollar, but without receiving the JOURNAL OF PARASITOLOGY.

6. *Honorary Life Members.* Upon unanimous vote, the Council may recommend that the society confer honorary life membership upon distinguished American parasitologists over sixty years of age. Honorary Life Members shall enjoy full membership privileges and shall be exempted from the payment of dues. The number of Honorary Life Members at any one time shall be limited to five.

7. *Foreign Honorary Members.* Upon unanimous vote, the Council at its annual meeting may elect Foreign Honorary Members. No two men from the same country shall be elected in the same year. The number of Foreign Honorary Members at any one time shall be limited to

twelve. Foreign Honorary Members may receive the JOURNAL OF PARASITOLOGY upon payment of membership dues.

PRESENTATION OF PAPERS AT ANNUAL MEETINGS

1. Except for invited papers, and papers coming to the American Society of Parasitologists program through joint sessions with another society or section, all persons presenting papers must be members of the society in good standing, or must be introduced by a member in good standing.

2. Each member of the society in good standing may be allotted not more than fifteen minutes of program time, to be used in person or by a non-member introduced by him. If the program is crowded, the maximal allotment of time may be reduced to ten minutes.

3. Papers offered for presentation too late to be included on the printed program may be presented at the conclusion of any of the scientific sessions provided that the presiding officer obtains the consent of the members present.

OFFICERS

1. The Council shall act as a nominating committee and at the annual meeting shall submit to the society at least one nominee for each office to be filled. The Secretary shall invite the members of the society to submit nominations for consideration by the Council.

2. The Treasurer of the society shall act also as Treasurer for the JOURNAL OF PARASITOLOGY and shall be bonded for the sum of two thousand dollars.

3. A sum of fifty dollars shall be allotted annually to the Secretary, to the Treasurer, and to the Chairman of the Editorial Committee for attendance at the annual meeting.

MANAGEMENT OF THE JOURNAL OF PARASITOLOGY

1. The JOURNAL OF PARASITOLOGY, as the official organ of the society, shall be managed by an Editorial Committee appointed by the Council for a five-year period, and shall consist of one Protozoologist, one Helminthologist, and one Entomologist, one of whom shall be appointed by the Council to act as Chairman.

2. The Editorial Committee shall be assisted by an Editorial Board consisting of twelve members appointed by the Council for a four-year period in such a way that three members will retire and three new members shall be elected each year. The Editorial Board shall consist of two Entomologists, four Protozoologists, and six Helminthologists to be elected on the basis of attainment, interest in the society and geographical location.

3. The price of the JOURNAL OF PARASITOLOGY shall be five dollars per volume, except to members of the American Society of Parasitologists who shall receive it as a membership privilege included in the annual dues of four dollars.

ENDOWMENT FUND

1. Council shall select a Custodian of the Endowment Fund and two associates to whom it may delegate responsibility for management of the fund. The Custodian shall make an annual accounting to Council and such other reports as Council may request. The approval of two of the three custodians shall be necessary for the purchase, sale or exchange of securities. One of the three custodians shall be the Treasurer of the society and his signature shall be required on all vouchers of expenditure from the fund.

ADDITIONS AND AMENDMENTS

1. Additional by-laws may be created by a two-thirds vote of Council members present at a meeting, or by an affirmative vote of nine Council members in a ballot conducted by mail. By the same procedure existing by-laws may be repealed, amended or suspended.

AMERICAN SOCIETY OF PARASITOLOGISTS

Notice of Election of Officers

The Council of the American Society of Parasitologists, having voted to suspend annual meetings of the Society until travel restrictions are lifted, has recommended that President Henry E. Meleney appoint a Special Committee to select nominees for the offices of the Society for the year 1944. President Meleney has named the following members to this Committee: H. W. Stunkard (Chairman), F. C. Bishopp, and D. H. Wenrich. A ballot bearing the names of those persons nominated for office by the Special Committee is being sent to every member of the Society in good standing for his vote. On this ballot space will be available to permit members, if they wish, to vote for other persons besides those recommended by the Special Committee. It is urged that every member, whether or not he endorses the Special Committee's nominees, participate in the vote, returning at once the ballot, which is already stamped and addressed, to the Secretary.

JAMES T. CULBERTSON,
Secretary.

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